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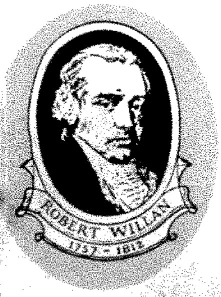

British Journal of Dermatology

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Ann Allergy. 1990; **64**: 191-194. 2. Ahn HS, Barnett A, Eur J Pharmacol, 1986; **127**: 153-155.

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REFERENCES

1. Boyle J, Burton JL, Faergemann J. Br Med J 1986; 292:28
2. Data on File. Scotia Pharmaceuticals Ltd.

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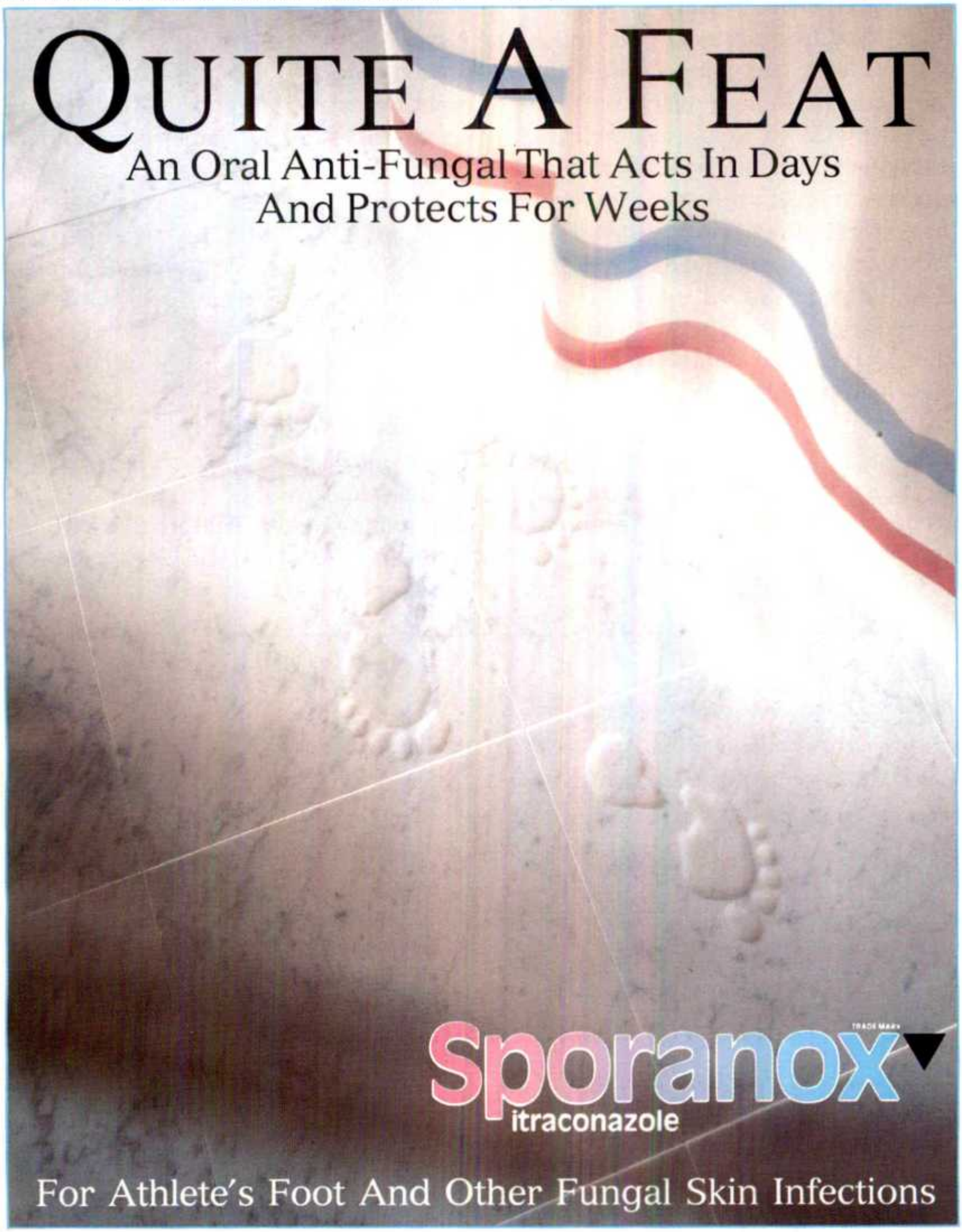
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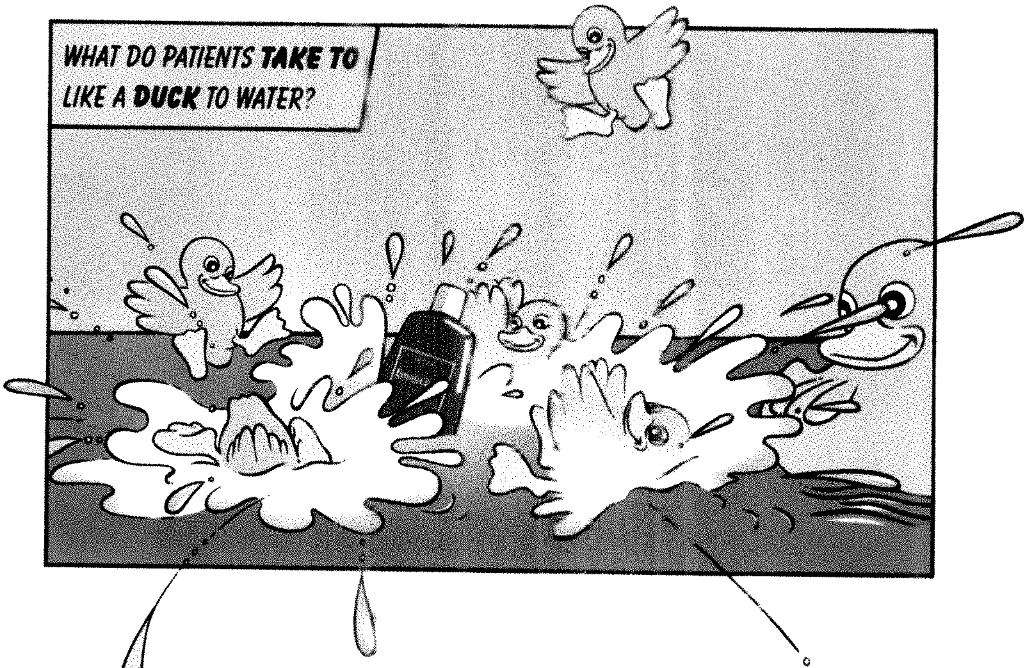
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Effect of topical retinoic acid on the interleukin 1α and β immunoreactive pool in normal human epidermis

D.CHATELLARD GRUAZ, L.DIDIERJEAN, D.GUMOWSKI-SUNEK AND J-H.SAURAT

Department of Dermatology, University Hospital, Geneva, Switzerland

Accepted for publication 3 April 1990

SUMMARY

The topical application of 0·1% retinoic acid (RA) on human skin over a period of 4 days, whether or not under occlusion, did not increase either IL-1α or β immunoreactivity as determined by a sensitive enzymoimmunoassay. No down modulation was seen following the application of a potent topical corticosteroid. Occlusion increased the yield of IL-1β immunoreactivity. Immunoblot patterns of epidermal extracts revealed both the mature form of IL-1 (17 kDa) and the precursor (36 kDa) and were identical in amounts whether the specimens were from controls or from RA- or corticosteroid-treated skin. There was a slight modification in the pattern of high molecular weight proteins (52 kDa) probed by the anti-IL-1α and β sera. It appears that the IL-1 epidermal immunoreactive pools are barely amenable to modulation because they represent a storage form linked to end-stages of keratinocyte differentiation.

Topical retinoic acid (RA) is widely prescribed in the treatment of various disorders. It was initially used in the treatment of acne vulgaris[1] and more recently as a therapy for sun-damaged skin.[2] The effects of topically-applied RA may involve cytokines in the epidermis.[3,4] We have found that normal human epidermis contains significant amounts of the biologically active interleukin 1 (IL-1).[5] The epidermal pool of IL-1 is increased in the rat during systemic administration of acitretin, a synthetic retinoid that mimics the biological effects of natural RA.[4] Whether this also occurs in humans after topical application of RA is unknown. We have studied the effect of the topical application of 0·1% RA on the levels of two forms of IL-1 (IL-1α and β) in human epidermis, as determined by a sensitive and specific enzymoimmunoassay (EIA). For comparison we also tested the effect of a potent topical corticosteroid.

Correspondence: Professor J-H. Saurat, Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH-1211 Geneve 4, Switzerland.

METHODS

Topical application of retinoic acid and steroid

All the experiments were carried out on healthy human volunteers aged between 16 and 56 years (mean 28·9 ± 0·8 years). Informed consent was obtained and the study was approved by the local Ethical Committee. To the right and left buttocks were applied 100 µl of 0·1% retinoic acid (RA) or solvent, each to an area of about 4 cm^2 as previously described.[6] These sites were occluded and the procedure was repeated over a period of 4 days. On the fifth day epidermal biopsies were taken from both sides under local anaesthesia with a keratotome set at about 180 µm. The biopsy samples were immediately frozen in liquid nitrogen. In some experiments the same procedure was followed except that the occlusive dressing was not applied. Difluorocortolone valerate 0·1% in a vanishing cream or the cream base was applied under occlusion to some subjects in the same way as with the RA.[6]

To check for variation in IL-1 content for various sites, normal skin samples were obtained with a keratotome from the breast and abdomen of five women undergoing plastic surgery. Since these skin samples contained about 20% of the upper dermis,[6] pure normal epidermis obtained from suction blisters from controls was also used. These suction blisters were raised on the anterior aspect of the thigh of four healthy volunteers using a negative pressure of 200 mmHg for 120–150 min.[5]

Preparation of epidermal extracts

Proteins were extracted by homogenization using a Polytron and by sonication three times for 15 s in phosphate-buffered saline with 20 mM EDTA and 5 mM iodoacetamide. The supernatants obtained by centrifugation at 10,000 *g* for 15 min were used for measurement of total protein (Biorad), immunoblotting and EIA.

SDS-PAGE and immunoblotting

For immunoblotting, sheep antiserum directed against human recombinant IL-1β and a goat antiserum directed against recombinant human IL-1α were used as previously described.[7] The proteins (40–50 µg) were run in 10% SDS gels and protein blots were performed.[7] The sheets were stained with Ponceau red, photographed and then saturated at room temperature for 2 h in 10 mM Tris-HCl containing 3% BSA at pH 4. The sheets were then incubated with antisera diluted 1/100 in 10 mM Tris-HCl, 0·5% Nonidet P40, 0·3 M NaCl, 3% BSA at pH 7·4 for 2 h. After washing five times for 5 min in the same buffer without BSA, the sheets were incubated for 1 h with biotinylated rabbit antisera directed against sheep or goat IgG (ABC kit, Vector Laboratories, Burlingame, CA, U.S.A.) diluted 1/2000 in the buffer and followed by incubation with avidin-HRP complex. Final staining was with diaminobenzidine and H_2O_2. The specificity controls were carried out as previously reported.[7]

Enzymoimmunoassay (EIA)

IL-1α and IL-1β were measured by EIA.[8] This is a conventional immunometric assay using tandem monoclonal antibodies. One hundred microlitres of either sample or standard (recombinant human IL-1α or IL-1β) were placed into the wells of a microtitre plate that were coated with a monoclonal antibody specific for either IL-1α or IL-1β before 100 µl of a second monoclonal antibody coupled to acetylcholinesterase was added. After incubation overnight at 4°C, the plates were washed and 200 µl of substrate solution (Ellman's medium) were placed into each well to measure the acetylcholinesterase activity bound on the solid phase. After 30 min of enzymatic reaction, the absorbance at 405 nm was measured. The detection limit of the assay is below 10 pg/ml of IL-1α or IL-1β. No significant cross-reactivity was observed between IL-1α and IL-1β.[8]

RESULTS

Interleukin 1α and β in normal human skin

EIA. Table 1 shows that the amounts of IL-1α and β are similar in the pure epidermis obtained from suction blister tops and in 180 μm specimens obtained with a keratotome. This indicates that in normal skin the superficial dermis does not significantly contribute to the EIA values. IL-1α was readily detectable in all the specimens tested in the range of ng/mg protein, whereas IL-1β was under the limit of detection of the assay (< 10 pg/ml).

Immunoblotting. In untreated skin, whether it was occluded or not, the principal IL-1α immunoreactive protein was observed as a doublet in the 55–52 kDa range (Fig. 1a, lanes 2, 4, 6). Similarly IL-1β immunoreactive 52 kDa bands were also observed (Fig. 1b, lanes 2, 4, 6) in samples where no IL-1 protein was detected. This is not due to the epitope specificity of the antibodies used in the EIA since the tandem of monoclonal antibodies involved in this assay also recognizes the 52 kDa IL-1β band. Faint 36 kDa (precursor) immunoreactivities were revealed with both anti-IL-1α and β sera, whereas the 17 kDa (mature form) was only detected with the anti-IL-1α serum (Fig. 1a, lanes 2, 4, 6).

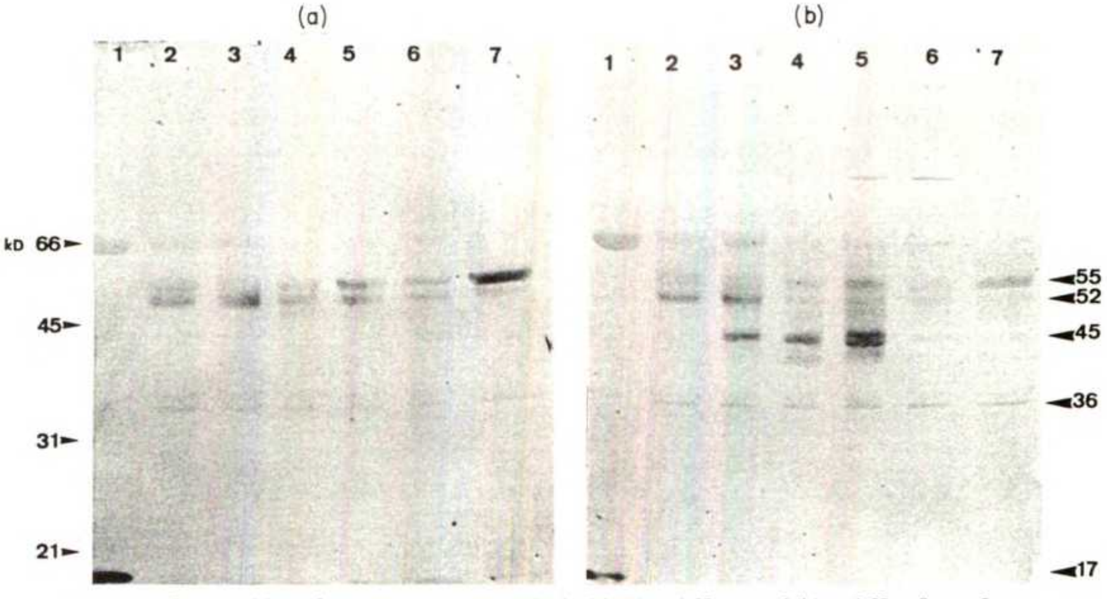

FIGURE 1. Immunoblots of cutaneous extracts probed with (a) anti-IL-1α and (b) anti-IL-1β sera. Lane a1 contains 25 ng of recombinant human IL-1α and lane b1 25 ng recombinant human IL-1β. Both recombinants were diluted in PBS containing 1% BSA. Lanes 2–5 correspond to the RA experiments. Lanes 2 (control) and 3 (RA) under occlusion, lanes 4 (control) and 5 (RA) without occlusion. Lanes 6 (control) and 7 (steroid) correspond to the steroid experiments under occlusion. Small arrows indicate the migration of standard molecular weight markers: 66 kDa, BSA; 45 kDa, ovalbumin; 31 kDa, carbonic anhydrase; 21 kDa, soybeantrypsin inhibitor. Large arrows indicate the molecular weight of immunoreactive proteins.

Effects of topical application of retinoic acid (RA)

EIA. Figure 2a shows that the values of IL-1α obtained after treatment with RA, whether with occlusion or not, are higher than those of the untreated controls. However, a statistical significance was not obtained due to a considerable variation within the experimental group ($P = 0·500$). There was no difference between the values of RA-treated skin whether with occlusion or not, and with those specimens treated with the solvent only.

There was no difference between the IL-1β content of untreated skin as compared with either the RA- or solvent-treated skin without occlusion, as shown in Figure 2b. The IL-1β values were very low, usually below the detection threshold of the assay. In the specimens treated with occlusion there was a higher content of IL-1β. There was no difference between the RA- and solvent-treated sites with occlusion.

Immunoblotting. Topical treatment with RA enhanced the 55–52 kDa IL-1α signal (Fig. 1a, lanes 3, 5) and induced a 45 kDa IL-1β band (Fig. 1b, lanes 3,5). Such a 45 kDa immunoreactivity has been described in LPS-stimulated monocytes,[9,10] and this is immunologically unrelated to the bioactive IL-1 17 kDa form. The 36 and 17 kDa forms were not altered by topical treatment with RA.

Effects of topical application of difluorocortolone valerate

EIA. The topical application of corticosteroid under occlusion did not significantly alter either the IL-1α (Fig. 3a) or IL-1β (Fig. 3b) content in the epidermis. Probably as an effect of occlusion, there was more IL-1 in the treated than in the untreated skin specimens. However, the amounts of IL-1β recovered from these specimens were much lower than those recovered from the epidermis treated with RA, 20 and 17 pg/mg protein versus 110 pg/mg protein (Figs 3b and 2b, respectively). This is possibly due to the use of a different vehicle in the steroid experiment. When calculated either as per ml or per µg DNA, the levels of IL-1 were similar to those expressed as per mg protein in all the groups tested (data not shown).

Immunoblotting. Treatment with topical steroid enhanced the 55–52 kDa IL-1α and IL-1β immunoreactivities (Figs 1a and b, lane 7). There were no variations of the 36 and 17 kDa IL-1 forms.

TABLE 1. Enzymoimmunoassay determination of IL-1α and β in the normal skin of untreated control subjects

	ng/mg protein (± SEM)	
	IL-1α	IL-1β
Suction blister top (pure epidermis) $n = 4$	7·29 (±4·69)	< 0·001 —
Keratotome specimens (epidermis plus 20%, superficial dermis) $n = 5$	9·57 (±2·28)	< 0·001 —

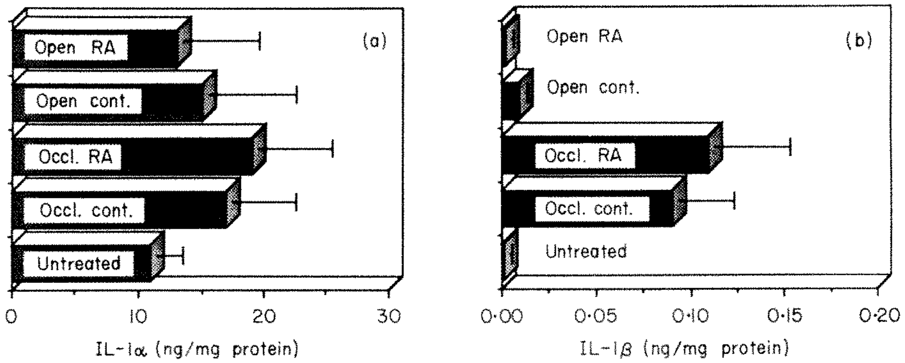

FIGURE 2. Enzymoimmunoassay values (ng/mg protein) for (a) IL-1α and (b) β: effect of topical retinoic acid (RA) treatment. Open RA, RA treated skin without occlusion ($n=4$); Open cont., vehicle treated control skin without occlusion ($n=4$); Occl. RA, occluded RA treated skin ($n=9$); Occl. cont., occluded vehicle treated control skin ($n=9$); Untreated, untreated normal human skin without occlusion ($n=5$).

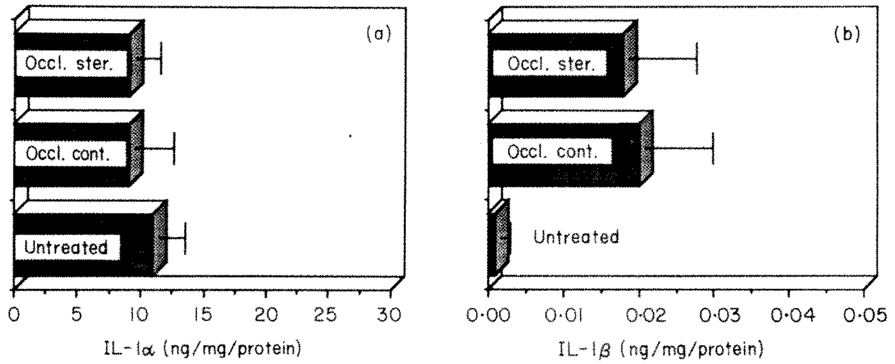

FIGURE 3. Enzymoimmunoassay values (ng/mg protein) for (a) IL-1α and (b) β: effect of topical difluorocortolone treatment. Occl. ster., occluded steroid treated skin ($n=6$); Occl. cont., occluded vehicle treated skin ($n=6$); Untreated, untreated normal human skin without occlusion ($n=5$).

DISCUSSION

The results of this study show that topical application of retinoic acid (RA) did not modify the amount of immunoreactive IL-1α and/or IL-1β proteins in human epidermis. This was not due to a lack of biological activity by RA following 4 days of topical application, because it has been previously shown under the same experimental conditions that there is already a significant rise of the level of cellular RA binding protein after 24 h.[6] The biological activity after 4 days of topical RA has also been shown (J.J.Voorhees, personal communication). Also, *in vitro* exposure of A431 keratinocytes to 10^{-10} M RA produces a decrease in both IL-1 mRNAs and proteins within 2 h.

IL-1α and β occur in two forms. The 17 kDa protein represents the mature, biologically active species that are secreted outside the cell after the enzymatic cleavage of a 36–31 kDa cytosolic precursor.[10] Identification of the 17 and 36–31 kDa forms by immunoblot analysis of epidermal extracts that are rich in both IL-1 bioactivity and EIA-immunoreactive IL-1, has been difficult.[7,11] However, higher molecular weight immunoreactive proteins of 55–52 and 45 kDa were consistently found.[7] Whether this results from the techniques used is currently under investigation. The present study showed that the topical application of both RA and steroid did not significantly alter the immunoblot pattern and the 36 and 17 kDa forms. There were some modifications of the higher molecular weight forms of IL-1 following treatment with RA and topical steroid.

The lack of quantitative modulation of IL-1 *in vivo* following the topical application of RA in humans contrasts with what was previously found in hairless rats given acitretin (RO 101670) intraperitoneally.[4] The biologically active IL-1 pool was increased after 3–5 days of treatment by 65%.

There are several possible explanations for the lack of modulation of IL-1α and IL-1β by topical RA. IL-1α appears to be the principal form in the epidermis, and significant amounts can be extracted from the normal unstimulated skin in the absence of any inflammation.[5,11] The reason for these high amounts is as yet unknown.[3] We have localized IL-1 immunoreactivity to the granular layer of the epidermis using immunoelectron microscopy.[7] Although the contribution of this localization to the extractable IL-1 is unknown, we suggest that the IL-1 epidermal immunoreactive pool represents a storage form linked to the end stage of differentiation of the keratinocyte.[7] Thus, any pulsed increase in the production of IL-1 would be barely detectable. This hypothesis is supported by the lack of effect of a potent topical steroid that should have decreased IL-1 epidermal levels if this was amenable to modulation. Corticosteroids have been shown to inhibit IL-1 production at the level of mRNA translation[12] and transcription.[13] Further studies of modulation of IL-1 by topical retinoids should therefore include techniques that allow the identification of newly synthesized IL-1 rather than the total amount that can be extracted.

IL-1β was significantly elevated in the specimens occluded *in vivo* as compared with those without occlusion. This effect might be due to an increased recovery of sweat in the specimens under occlusion. We have shown that sweat contains both IL-1α and β (approx. 32·4 ng IL-1α/mg protein and 5·6 ng IL-1β/mg protein as determined by EIA in the sweat from palms and soles).[8,14] The low constitutive level of IL-1β in the epidermis explains why the sweat-derived IL-1β might be a significant factor in the EIA values, whereas this is not so for IL-1.

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DNA content and $K_s8.12$ binding of the psoriatic lesion during treatment with the vitamin D_3 analogue MC903 and betamethasone

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SUMMARY

Twenty patients with psoriasis were treated with the vitamin D_3 analogue MC903 and betamethasone ointment in a double-blind trial with a left-right comparison. In addition to the clinical severity scores, $K_s8.12$ binding which detects keratin 16 expression and the DNA synthesis were quantified using flow cytometry. Both markers decreased significantly with treatment, but remained above the normal range even in those who had total clearance of the lesions. Treatment with MC903 with regard to $K_s8.12$ binding was significantly better than with betamethasone.

Psoriasis is a very common skin disorder that is characterized by increased epidermal cell proliferation. It is well established that vitamin D_3 (1α,25 dihydroxyvitamin D_3) inhibits epidermal cell proliferation in culture.[1] Vitamin D_3 is also claimed to be effective in the treatment of psoriasis.[2,3] A new vitamin D_3 analogue, MC903, has been found to be as potent as $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in inhibiting epidermal proliferation. It is, however, 100 times less active than $1\alpha,25\text{-(OH)}_2\text{-D}_3$ as regards its effect on calcium metabolism.[4] Therefore, it can be applied in higher concentrations than $1\alpha,25\text{-(OH)}_2\text{-D}_3$. It has also been shown in a double-blind study that MC903 in concentrations of 10 μg/g or greater is effective in the treatment of psoriasis.[5]

The aim of this study was to assess how effective MC903 was in the treatment of psoriasis as compared with betamethasone, by measuring binding of $K_s8.12$ and DNA content using flow cytometry.[6–8]

METHODS

Patients

Twenty patients (10 males and 10 females, age range 20–79 years) with chronic stable plaque psoriasis participated in this study after giving informed consent. The percentage body surface area involved with psoriasis ranged in these patients from 5–30%. In the 8 weeks before starting

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the trial none of the patients had systemic treatment for their psoriasis or ultraviolet light therapy. For at least 2 weeks before treatment no topical antipsoriatic therapy was used. Patients on systemic drugs and with renal disease, hepatic impairment or those with abnormal calcium metabolism were excluded from the study.

For 2 weeks the patients applied an emollient only to the affected areas. Then one side of the body was treated with MC903 ointment (50 mg/g) and the other with 0·1% betamethasone ointment. Both ointments were applied twice daily but not to the face, scalp and genital region. The study was a double-blind one.

During treatment the patients were assessed clinically and blood investigations were carried out at regular intervals. The efficacy of the treatments was assessed using the Psoriasis Area Severity Index (PASI) and clearance was assessed as complete resolution of the lesions apart from a residual discoloration.

Skin samples

Before treatment razor-blade biopsies (0·5 mm thick and 4 mm in diameter) were taken from two symmetrical lesions. After 6 weeks of treatment two further biopsies were taken from the same areas. Epidermal suspensions were prepared using trypsin as previously described[8] and these were fixed in 70% ethanol and stored at −20°C until used.

Analytical procedures

An aliquot of 500 μl of the cell suspension was rehydrated in phosphate-buffered saline (PBS) with 1% normal goat serum (NGS; Sera-lab, Sussex, U.K.) and labelled using an indirect-immunofluorescence technique.[6,9] The cells were incubated with a monoclonal antibody $K_s8.12$ (Sigma, St Louis, MO, U.S.A.) and diluted 1 : 30 in PBS/NGS. A second antibody, an FITC-conjugated goat anti-mouse IgG was used, which was diluted 1 : 25 in PBS/NGS. The sediment was resuspended in 200 μl PBS/NGS with 8 mg propidium iodide (Calbiochem, San Diego, CA, U.S.A.) and 50 μl 1% ribonuclease A (Sigma, St Louis, MO, U.S.A.) to measure the relative DNA content per cell[10] simultaneously with the green fluorescence of the $K_s8.12$ positive cells.

Five thousand cells from each sample were analysed using an Ortho 50H flow cytometer with a 5 W argon laser tuned at 488 nm as previously described.[6] The data were stored and analysed with a PDP 11/34 computer.[6,9,10]

Statistical analysis

A Wilcoxon rank test for paired data was used for the comparison of the results from the MC903 and betamethasone treated sites. *P* values less than 0·05 were considered to be significant.

RESULTS

Both MC903 and betamethasone ointments resulted in clearance of the lesions in eight of the 20 patients. A mild improvement was seen in five patients and a moderate one in seven. As with the clinical response, $K_s8.12$ binding and DNA content were reduced significantly ($P < 0·01$) as a result of both treatments (Figs 1a and b). The values in these three groups as determined by their clinical response to MC903 or betamethasone are shown in Table 1.[6,11]

Comparing the MC903-treated with the betamethasone-treated sites a lower final average K_s 8.12 binding value was found for MC903, although the difference was not statistically significant. However, a statistically significant difference was found in favour of MC903 during

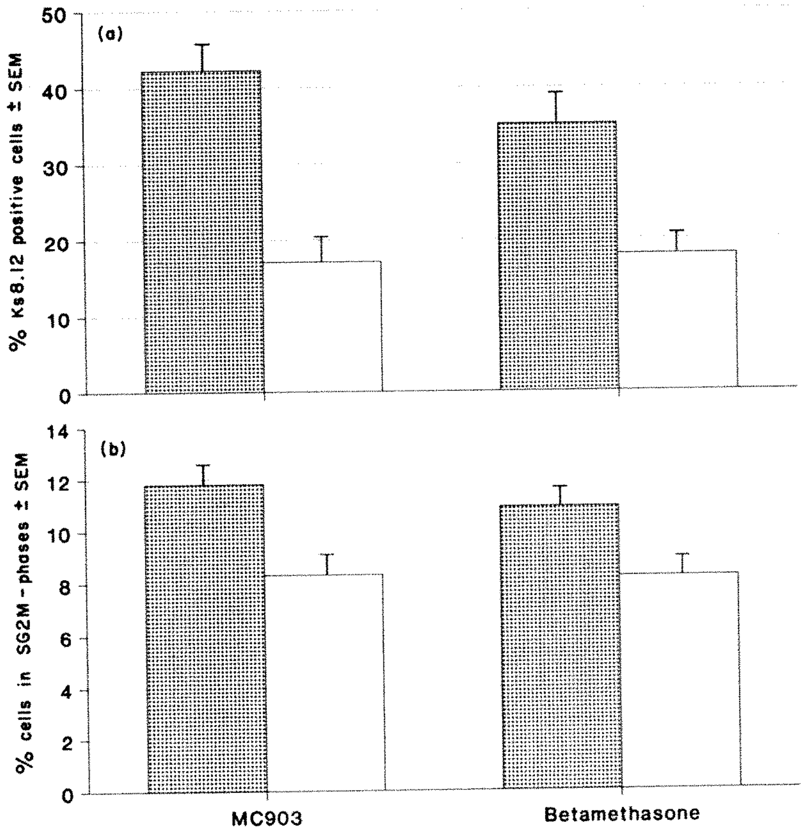

FIGURE 1. Markers for epidermal growth before treatment ▨ and after treatment ☐ (means ± SEM). (a) Percentage of K_s8.12 positive cells; (b) percentage of cells in SG_2M-phases.

TABLE 1. Mean (± SEM) values of parameters for epidermal growth in biopsies after treatment with MC903 or betamethasone. Patients are subdivided according to their clinical response. Values for normal skin and psoriatic uninvolved skin are included as a reference

Clinical appearance	% Ks 8.12 positive cells	% cells in SG2M-phase
Total clearance	8·4 ± 1·5	6·6 ± 0·4
Moderate improvement	19·5 ± 3·3	7·1 ± 0·6
Mild improvement	29·8 ± 6·8	12·4 ± 1·4
Normal skin	1·8 ± 1·0[6]	4·0 ± 0·7[11]
Uninvolved skin of psoriatics	1·0 ± 0·2[6]	5·6 ± 1·1[11]

treatment ($P < 0{\cdot}05$). The reduction in DNA content showed no significant difference between the two treatments. Following therapy, the patients that had clearing of the lesions had a significantly lower $K_s8.12$ binding ($P < 0{\cdot}03$) and DNA content ($P < 0{\cdot}05$) compared to the other patients. However, these parameters were still above the normal range.

DISCUSSION

This study confirms that the vitamin D_3 analogue MC903 is effective in the treatment of psoriasis.[12] It is at least as effective as betamethasone with regard to the effect on epidermal growth. The reduction of $K_s8.12$ binding and DNA content showed a positive correlation with the reduction of the PASI scores ($P < 0{\cdot}006$ and $P < 0{\cdot}03$, respectively). However, the values of these cell kinetic parameters were still above the normal range even in patients who showed total clearance. These findings have also been observed following treatment over 6 weeks with dithranol.[13]

It is possible that the level of binding of $K_s8.12$ and DNA content became normal when treatment was discontinued. However, both topical steroids and MC903 have a direct antiproliferative effect as has been shown *in vitro*.[1,4,14,15] An alternative explanation using cellular kinetics is that treatment was incomplete. It is possible that MC903 and betamethasone only apparently normalized the epidermis, leaving the basic defect of psoriasis unchanged. In the post-treatment follow-up to the trial, half the patients showed a reappearance of new lesions within 4 weeks. Whether further treatment after apparent clinical clearance might be associated with a longer remission period is uncertain. Continuation of treatment with PUVA or with UVB following clinical resolution has resulted in the prolongation of the remission period.[16–18] Further studies are indicated to determine whether maintenance treatment with MC903 will result in complete normalization of the cellular kinetic parameters of the epidermis and longer periods of remission of psoriasis.

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Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides

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SUMMARY

The role of locally released cytokines in inducing lymphocyte activation and infiltration in the skin lesions of mycosis fungoides has been investigated. The levels of selected cytokines were measured in chamber fluid samples from lesional and control skin. Biologically active interleukin 6 was significantly elevated in lesional samples and a recombinant form of this cytokine was shown to induce lymphocyte migration in an *in vitro* assay. Biologically active interleukin 1 was detected in all control chamber fluid samples. Significantly reduced levels of this cytokine were present in lesional samples, which may be the result of the release of preformed material. Interleukin 2 and tumour necrosis factor activity, and γ interferon and granulocyte macrophage colony-stimulating factor immunoreactivity, were not detectable in any of the samples. Interleukins 1 and 6 may play a role in the pathogenesis of the lesional lymphocyte infiltrates in mycosis fungoides.

Mycosis fungoides (MF) is a T-cell lymphoma in which the abnormal T cells are localized in the early stages to the skin.[1] In fully developed lesions, histology shows a dense lymphocytic infiltrate in the papillary dermis which extends into the epidermis. Groups of lymphocytes may invade the epidermis to form Pautrier microabscesses. In earlier lesions the type of cellular infiltrate in the upper dermis is more heterogeneous, and reactive lymphocytes, monocytes, histiocytes and eosinophils may be present. The local generation of factors which affect the migration and activation of lymphocytes and other cells may therefore play a critical role in the evolution of MF lesions.

The cytokine interleukin 1 (IL-1) has been recovered in biologically active amounts from epidermal samples obtained from normal human skin.[2–5] This evidence together with the

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evidence that IL-1 mRNA is present in cultured normal human keratinocytes[6] suggests that normal human epidermis is intrinsically capable of synthesis of IL-1 *in vivo*. However, it appears not normally to be available to target cells, possibly because of intracellular retention[7] or membrane association.[8] Probably for these reasons its potent biological properties, which include the ability to stimulate lymphocyte migration,[9–11] are not readily evident in normal skin. Reports of IL-1 levels in cutaneous MF are conflicting, one suggesting increased levels of IL-1 in MF lesions as determined by immunohistochemistry of skin biopsies,[12] the other indicating similar levels in chamber fluid samples from lesional and control skin as determined by a low-specificity murine-thymocyte co-stimulator assay.[13] To clarify this issue we have investigated the levels of biological activity of IL-1 in samples from lesional and from clinically uninvolved skin of patients with MF, using a more specific bioassay. The levels of other cytokines which may be relevant to lymphoproliferative and inflammatory disorders of the skin have also been determined. Interleukin 6 (IL-6) which is produced by epidermal cells *in vitro*[14,15] and which shares some biological properties with IL-1[16] has been measured and its chemoattractant properties in a lymphocyte-migration assay have been determined. The other cytokines assayed are the T-cell mitogen interleukin 2 (IL-2), which is produced by lymphocytes following stimulation by IL-1 and which stimulates lymphocyte migration *in vitro*;[17,18] tumour necrosis factor (TNF) which shares some biological properties with IL-1;[19]granulocyte-macrophage colony-stimulating factor (GM-CSF) which is produced by epidermal cells *in vitro*[20] and which may induce T-cell proliferation;[21] and γ interferon (γ-IFN) which enhances T-cell–keratinocyte interactions *in vitro*.[22]

METHODS

Patients

The diagnosis of MF was made on clinical grounds and confirmed by histopathological examination of a skin biopsy. The patients had plaque-type disease, stage IA, when graded according to the North American co-operative group.[1] Seven white Caucasian patients (five male) aged between 39 and 84 years, took part in the study after institutional Ethics Committee approval and informed consent had been obtained. The duration of MF was between 0·25 and 30 years (mean 19·5 years). At the time of the study four patients were applying bland emollients, one had applied 0·25% betamethasone valerate ointment until 2 days before sampling, and two had received maintenance photochemotherapy using 8-methoxypsoralen and UVA up to either 1 or 6 weeks beforehand.

Sampling methods

Exudate from lesional and clinically normal skin was collected using a previously described skin chamber technique.[23] The lesions selected were on the buttocks, lower back, thighs or abdomen. Clinically normal skin was sampled at the symmetrical site on the opposite half of the body in all cases except one, when skin adjacent to the lesion was sampled. Briefly, skin was abraded gently with a scalpel blade over areas 2 cm in diameter until a glistening surface appeared. Sterile acrylic cylinders were fixed to the abraded sites with cyanoacrylate adhesive and filled with 1 ml sterile phosphate-buffered saline (PBS). This was removed after 2 min and discarded, so as to reduce any effects of abrasion on cytokine levels. A further 1 ml PBS was added, collected after 60 min, mixed with 10 U of preservative-free heparin and stored at −20°C.

Cytokine assays

Interleukin 1 was measured in a specific, two-stage bioassay (detection limit approximately 200 fg/ml) incorporating the murine thymoma line EL-4 NOB-1 and CTLL cells, as previously described.[24] The assay does not detect lipopolysaccharide, α-, β- and γ-IFN, IL-2, GM-CSF, G-CSF, M-CSF, transforming growth factor β or interleukins 3, 4, 5, 6 and 8 in concentrations up to 1 μg/ml. TNF α and β induce suboptimal responses in concentrations above 10 ng/ml[24] (A.J.H.Gearing, unpublished observations). IL-2 activity was measured by the proliferative response of the IL-2-dependent cell line CTLL, in an assay capable of detecting approximately 500 pg IL-2/ml.[24] IL-6 levels were determined by using the B9 cell-proliferation assay[25] and TNF by a cytotoxicity assay incorporating actinomycin D-treated L929 cells[26] (detection limits approximately 500 fg/ml and 20 pg/ml, respectively). GM-CSF levels were measured by two-site Insight GM ELISA (Medical Resources Limited, Surry Hills, New South Wales, Australia; detection limit approximately 10 pg/ml) and γ-IFN by two-site immunoradiometric assay (Centocor, Malvern, PA, U.S.A.; detection limit approximately 20 pg/ml).

Lymphocyte migration assay

Lymphocyte migration in response to IL-6 was assessed using mixed peripheral blood lymphocytes containing < 1% monocytes in a 48-well microchemotaxis chamber as previously described.[27] The lymphocytes were obtained from normal subjects. In brief, 25 μl of chemoattractant in the lower wells of the chemotaxis chamber was separated from 50 μl of cell suspension (2×10^6 cell/ml) in the top wells by an 8 μm pore-size polyvinylpyrrolidone-free polycarbonate filter. Following incubation for 1 h at 37°C the filter was removed. Cells on the top side were wiped clean and cells on the undersurface fixed and stained for quantitation by image analysis. The results were expressed as a migration index (area of lower surface of filter occupied by stimulated cells (mm^2) divided by the area (mm^2) of lower surface of filter occupied by unstimulated cells).

The lymphocyte subsets migrating in response to IL-6 (10^{-9} M) were compared with those migrating in the presence of medium alone, by immunocytochemical analysis of cells adherent to the lower surface of polycarbonate filters following a standard assay with mixed peripheral blood lymphocytes. Following incubation, the filters were removed, attached to glass slides, air-dried for 3 h, then fixed in acetone/methanol (1 : 1 by vol) for 90 s. Following a 10-min wash in Tris-buffered saline (TBS, 0·05 M, pH 7·6), rabbit serum (1/20 dilution in TBS, Dako, High Wycombe, U.K.) was applied to the filters for 20 min. The appropriate primary antibody, optimally diluted, was then applied and the slide left in a moist chamber at 4°C for 18–20 h. The slide was then washed with TBS and link antibody (rabbit anti-mouse immunoglobulin, code Z259, Dako), and diluted 1/25 in the above rabbit serum preparation, added for 30 min. Following a TBS wash, soluble complexes of alkaline phosphatase/monoclonal mouse anti-alkaline phosphatase (APAAP; Dako) were added in accordance with the manufacturer's instructions and as previously reported.[28] After 30 min, the slide was washed in TBS and the link antibody and APAAP steps repeated. Following reaction with naphthol AS-MX phosphate and Fast Red for 60 min,[28] slides were washed and counterstained with haematoxylin. The number of positively staining cells per five power fields was determined. Cytospin preparations of the mixed lymphocyte suspensions used in the migration assay were also stained and counted in the same way.

The primary antibodies used were Dako T4 (1/200 dilution), Dako T8 (1/800 dilution) (Dako, High Wycombe, U.K.), Royal Free Pan T (undiluted) and Royal Free Pan B (undiluted) (Academic Department of Immunology, Royal Free Hospital, London).

Assay standards

Non-glycosylated recombinant reference reagents from the National Institute for Biological Standards and Control, Hertfordshire, U.K. were used as standards in the described assays. They included IL-1 α (1 U ≈ 10 pg), TNF α (1 U ≈ 25 pg), IL-6 (1 U ≈ 200 pg), GM-CSF (1 U ≈ 10 pg), IL-2 (1 U ≈ 7 ng) and γ-IFN (1 U ≈ 30 pg). The biological assays were calibrated with a range of concentrations of standard reagents and chamber fluid samples assayed in duplicate after doubling dilutions from 4- to 1024-fold. Dilutions of chamber fluid causing responses similar to those in the middle of the standard concentration-response curves were used for quantification. ELISA assays were carried out in accordance with the manufacturers' instructions.

RESULTS

IL-6 activity was detectable in all chamber fluid samples from MF lesions (Table 1), the mean concentration (353 ± 137 pg/ml) being significantly higher than in samples from clinically normal skin (22 ± 9 pg/ml; mean ± SE, $n = 7$; $P < 0{\cdot}05$, Wilcoxon signed-rank test).

IL-1 activity was found in chamber fluid samples from both clinically normal and lesional skin (Table 1). The mean concentration in lesional samples (208 ± 78 pg/ml) was significantly lower than in samples from normal skin (627 ± 237 pg/ml; mean ± SE, $n = 7$; $P = 0{\cdot}05$, Wilcoxon signed-rank test). IL-2 and TNF biological activity, and GM-CSF and γ-IFN immunoreactivity, were below the limit of detection whether from lesional or control skin.

The stimulation of *in vitro* lymphocyte migration by recombinant IL-6 is shown in Figure 1. Dilution-related responses were seen between 10^{-15} and 10^{-8} M, the latter being the highest concentration tested. The subpopulations of cells counted on filters after migration in response to IL-6 (10^{-9} M) and medium alone are shown in Table 2.

TABLE 1. Interleukin 6 and interleukin 1 (pg/ml) in chamber fluid exudate in involved (I) and clinically uninvolved (CUI) skin of seven patients with mycosis fungoides

	Interleukin 6		Interleukin 1	
Patient no.	CUI	I	CUI	I
1	13	22	240	240
2*	< 1†	1100	1280	800
3‡	< 1†	310	236	12
4	< 1†	17	1952	160
5	39	390	256	112
6	65	320	22	9
7	32	310	400	120
Mean ± SE	22 ± 9	353 ± 137	627 ± 237	208 ± 78

* PUVA was stopped 1 week prior to sampling.

† In calculating mean ± SE, values < 1 taken as 1.

‡ Topical corticosteroids were stopped 2 days prior to sampling.

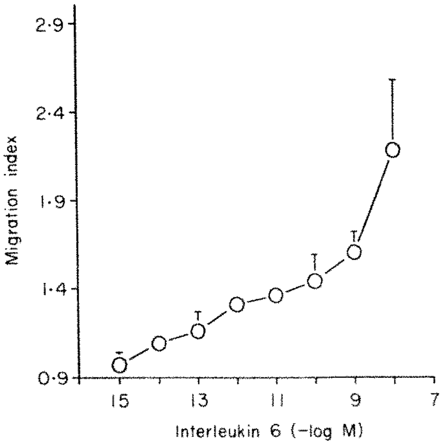

FIGURE 1. Dilution-related lymphocyte migration in response to recombinant interleukin 6. Each point on the graph represents mean ± SE of the migration index for three independent duplicate experiments. Error bars that are not shown were smaller than diameter of the symbols.

TABLE 2. Number of positively stained cells on the undersurface of filters in response to IL-6 (10^{-9} M) and medium alone, and subpopulations of cells in cytospin preparations of mixed lymphocyte suspensions used in the migration assay

Antibody	Cell specificity	IL-6 (10^{-9} M)*	Medium*	Cytospin preparations†
Pan T	T cell	261 ± 32	102 ± 4	91 ± 2
Pan B	B cell	13 ± 2	13 ± 4	7 ± 4
Dako-T4	T helper/inducer	135 ± 17	68 ± 4	73 ± 6
Dako-T8	T suppressor/cytotoxic	117 ± 14	30 ± 4	35 ± 2

* Number of positively stained cells per five high power fields (mean ± SE, $n = 3$).

† Number of positively stained cells, expressed as a percentage of total cells (mean ± SE, $n = 3$).

DISCUSSION

Interleukin 6 (IL-6) is produced by epidermal cells,[14,15] fibroblasts[29] and endothelial cells[30] *in vitro*. It has also been reported to be detectable in elevated levels in psoriatic lesions by use of immunohistochemical methods,[31] although analysis of lesional psoriatic stratum corneum samples failed to demonstrate bioassayable amounts.[32] To our knowledge increased IL-6 biological activity in the cutaneous lesions of MF has not been reported before. However, in view of the ready *in vitro* production of IL-6 by keratinocytes, fibroblasts and endothelial cells, it seems unlikely that its *in vivo* production will be specific to any one skin disease. IL-6 is capable both of activating T cells[16] and of inducing human lymphocyte migration (both $CD4^+$ and $CD8^+$ cells), as demonstrated here. The levels of IL-6 which were measured in lesional skin chamber fluid would be weakly chemoattractant to lymphocytes *in vitro* (Fig. 1). However, preliminary findings (K.B.Bacon, unpublished observations) indicate that there is approximately 100-fold dilution of exudate by PBS in the skin chamber sampling method. Accordingly, *in vivo* levels in the skin are considerably higher than those recovered in skin chamber fluid and may influence lymphocyte migration in the concentrations which are present. IL-6 should therefore be considered as a mediator of potential importance in the induction of lymphocyte infiltrates in MF and possibly other skin diseases. Immunocytochemical analysis has shown that it may influence the migration of both T-helper and T-suppressor cells. However IL-6 did not influence neutrophil migration in an agarose microdroplet assay (N.F.Fincham, personal communication).

The presence of IL-1 activity in chamber fluid samples from the clinically normal skin of patients with MF is in keeping with previous reports of biologically active amounts of IL-1 in normal epidermal extracts.[2-5] The lower levels of IL-1 activity found in samples from MF lesions suggest either that IL-1 production in the lesions is decreased or that the disease process leads to utilization, inhibition or release of preformed IL-1 and consequent decreased detectable levels. That IL-1 is readily released from the clinically normal skin of MF patients has been shown by the skin chamber sampling method used in the present experiments. Similar findings have been reported in psoriasis, where lesional epidermal samples contain lower levels of IL-1 than those from normal skin.[5,33,34]

Tron *et al.*[12] used an anti-human IL-1β monoclonal antibody to perform direct immunofluorescence studies on frozen sections of biopsies from normal skin and MF lesions. Biopsies from MF lesions showed strong intercellular immunoreactivity in the epidermis, whereas normal tissue did not show intercellular labelling. This contrasts with our results and can be explained by the more recent evidence which indicates that IL-1-α-like material is the major biologically active IL-1 component in human epidermis.[6,35] An immunoreactive but biologically inactive IL-1-β-like substance has been recovered both from psoriatic lesions and normal skin.[32,35] The presence of this substance may explain the positive immunofluorescence found in Tron's study.[12] Dowd *et al.*[13] reported the presence of similar amounts of IL-1-like activity in chamber fluid samples from the involved and uninvolved skin of three MF patients using a non-specific mouse thymocyte co-stimulator assay. This assay is unable to discriminate between IL-1, IL-2, IL-6 and other cytokines and these results may have been due to the combination of increased IL-6 with decreased IL-1 in MF lesions compared to clinically normal skin.

The cause and relevance of the reduced levels of IL-1 biological activity in lesional MF samples are currently uncertain. However, if the reduced levels reflect the release of preformed material, both this and the finding of increased IL-6 activity raise the possibility that these cytokines play a role in the pathogenesis of lesional lymphocyte infiltrates in MF. Within the detection limits of the assays used, we have been unable to uncover evidence of involvement of IL-2, TNF, GM-CSF or γ-IFN in the pathogenesis of MF.

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Elastic fibre damage induced by low-dose D-penicillamine

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SUMMARY

We have studied 23 patients receiving penicillamine for the treatment of rheumatoid arthritis to determine the prevalence of penicillamine-induced elastosis. One female patient had pseudoxanthoma elasticum-like skin changes and bramble-bush elastosis without calcification in the involved skin. Penicillamine elastosis was present in the joint capsule in 62% of eight patients or 64% of 11 joints examined and was detected in joint capsules after as little as 1 year of treatment.

D-penicillamine induces a unique change in elastic tissue characterized histologically by small protrusions perpendicular to the long axis of elastic fibres giving them the so-called 'bramble-bush' or 'lumpy-bumpy' appearance.[1,2] 'High-dose' penicillamine therapy (2–3 g daily), as used for the treatment of Wilson's disease and cystinuria, may cause this type of elastic-fibre damage. If the dermal elastic tissue is affected, this may be associated with a variety of cutaneous problems. Skin fragility, elastosis perforans serpiginosa (EPS), cutis laxa, pseudoxanthoma elasticum-like change (pseudo-pseudoxanthoma elasticum)[3–5] and anetoderma[6] have been described in patients on 'high dose' penicillamine therapy. These elastic fibre changes may also be detected in clinically normal skin in patients taking penicillamine.[7] The amount of penicillamine required to produce elastic fibre changes is unknown, but it has been estimated that a minimum of 1·0 g daily for more than 5 years is necessary to induce EPS.[5] D-penicillamine is also used for the treatment of rheumatoid arthritis, but generally a 'low-dose' regimen (0·25–1 g daily) is employed. Penicillamine-induced elastic tissue damage had not been reported in these patients until two of our group (SMB and TJR) described a patient with rheumatoid arthritis in whom widespread systemic as well as cutaneous elastic fibre damage had been induced by low dose (0·75 g daily) penicillamine therapy.[8] Penicillamine elastosis was present in a joint capsule removed after 7 years' therapy and later the patient developed pseudo-pseudoxanthoma elasticum with characteristic 'bramble-bush' elastic fibre damage in the involved skin. 'Bramble-bush' elastosis was also detected in the adventitia of the viscera, the alveolar septa of the lung and the wall of the aorta and the authors concluded that even low-dose

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regimens may cause widespread damage to elastic tissue.[8] We have studied a further 23 patients receiving penicillamine for the treatment of rheumatoid arthritis to determine the prevalence of penicillamine-induced elastosis in skin and, when possible, joints.

METHODS

Patients

The patients were recruited from the Rheumatology Unit, Nuffield Orthopaedic Centre, Oxford. Twenty-three Caucasian patients receiving penicillamine for sero-positive rheumatoid arthritis gave informed consent. The study had the approval of the Oxford Research Ethics Committee. Details of penicillamine therapy were obtained from the flow chart in the medical records and the total dose of penicillamine was calculated for each patient.

Dermatological assessment

Skin type was assessed, the patients were questioned about skin fragility and examined for evidence of penicillamine-associated skin changes, e.g. milia, papules, bullae, EPS, pseudoxanthoma elasticum.

Ophthalmological assessment

Six patients consented to have ophthalmological assessment. A history of visual symptoms was taken and after pupillary dilatation, the eye was examined with the indirect ophthalmoscope. The retina was examined for the presence of angioid streaks.

Histology

Skin. Seventeen patients gave consent for a 4-mm punch biopsy of skin to be taken from a non-sun-exposed site on the inner upper arm and one patient for a biopsy to be taken from fragile, blistering skin on the dorsum of the hand. A similar biopsy was taken from the neck of a patient with clinical evidence of pseudoxanthoma-like change. The biopsies were processed routinely for histological examination and stained with orcein for examination of the elastic fibres.

Joint specimens. Tissue was also available from surgical procedures on the rheumatoid joints of eight patients (11 joints). Fifteen joint specimens from patients with rheumatoid arthritis who had never received penicillamine were also studied. The specimens were stained with orcein and reviewed independently by two observers (KLD and SMB), without knowledge of the patient's treatment, for evidence of elastic tissue damage.

RESULTS

Penicillamine therapy

The patient details together with duration of treatment and cumulative dose of penicillamine are shown in Table 1. Nine of the patients had received penicillamine for 10 or more years. Twenty-two patients had received doses between 0·25 and 1 g daily. One man with severe long-standing disease had received between 1·25 and 2·0 g daily for a period of 5·6 years, but had taken a small dose of 0·5 g daily for the 5 years prior to the study. The subgroup of 18 patients who consented to skin biopsy were aged from 24 to 72 years (mean 45·1 years) and had received penicillamine for between 2 and 15 years (mean 8·6 years).

TABLE 1. Sex, age, duration and dosage of therapy and skin biopsy results in 23 patients receiving penicillamine

Patient M/F	Age (years)	Duration of penicillamine treatment (years)	Cumulative dose (g)	Skin elastic tissue
1 M	63	10	1259	Not examined
2 M	61	2	425	Arm—normal
3 F	50	11	1820	Arm—normal
4 M	54	15	4800	Arm—normal
5 M	50	7	1686	Arm—normal
6 M	43	10	2775	Arm—normal
7 F	72	5	290	Arm—normal
8 F	32	8	887	Arm—normal
9 M	53	12	2184	Arm—normal
10 M	41	11	3624	Not examined
11 M	59	9	1579	Not examined
12 F	37	12	2570	Arm—normal
13 F	53	9	1319	Arm—normal
14 F	25	9	1524	Arm—normal
15 M	39	4	278	Arm—normal
16 M	68	6	932	Not examined
17 F	53	10	774	Arm—normal
18 F	32	11	499	Arm—normal
19 F	30	8	1691	Arm—normal
20 M	59	7	1016	Arm—normal
21 M	24	6	1103	Hand—normal
22 M	55	3	364	Not examined
23 F	45	9	2624	Arm—normal Neck—'Bramble-bush' elastosis

Dermatological examination

One male patient had marked fragility and blistering on the dorsum of the hands. The patient was taking naproxen and clinical, histological, immunofluorescence and biochemical findings were consistent with a diagnosis of naproxen-induced pseudoporphyria.

Pseudoxanthoma elasticum-like change with yellowish-white linear plaques in the skin creases of the neck (Fig. 1) was detected in a 46-year-old female patient. The skin in the axillae and groins was normal. She had received 0·25–0·75 g of penicillamine daily for 9·5 years (cumulative dose 2623 g). She was of skin type 1 (always burns but never tans) but gave a history of only mild recreational sun exposure.

Ophthalmological examination

The six patients were aged 31–64 years (mean 53 years) and they had received penicillamine for between 1 and 16 years (mean 9 years). One patient had pseudoxanthoma elasticum-like change. Five of the six patients examined had no visual symptoms. One described microscotomata and this was demonstrated bilaterally using an Amsler chart. This patient was found to have macular drusen (retinal colloid bodies most commonly associated with macular degeneration) and unrelated to penicillamine therapy. The visual acuity was 6/6 or better in all the eyes tested. Angioid streaks were not detected.

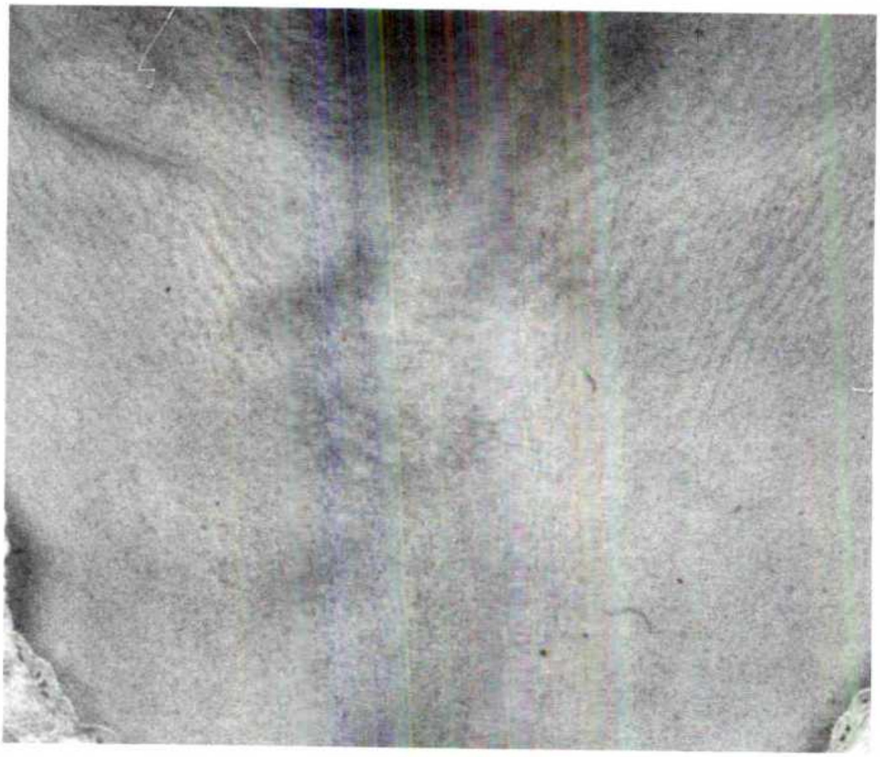

FIGURE 1. Yellowish-white plaques around neck of patient with pseudo-pseudoxanthoma elasticum.

Histology

Skin. Seventeen patients gave consent for a 4-mm punch biopsy of skin to be taken from a non-sun-exposed site on the inner upper arm, and one patient gave consent for a biopsy to be taken from the dorsum of the hand. No abnormal elastic tissue was found in these biopsies. A similar biopsy was taken from the neck of a patient with clinical evidence of pseudoxanthoma-like change. Bramble-bush elastosis was present in this skin (Fig. 2). There was no calcification of elastic fibres.

Joints. Penicillamine-induced elastic fibre damage was present in the tissue of the joint capsule in five of the eight patients examined (seven of 11 joints) (Table 2, Fig. 3). In one patient, joint specimens were available before starting penicillamine, after 1 year (568 g) of therapy and after 11 years (4800 g) of penicillamine therapy. No elastic fibre damage was evident before treatment but characteristic 'bramble-bush' elastosis was evident in the joint capsule after 1 year of penicillamine and this was more marked after 11 years of therapy. In another patient, penicillamine-induced elastosis was present in the capsule of an elbow joint after 2 years treatment but was not found in the knee joint 4 years later, despite continuous therapy. There was no 'bramble-bush' elastosis in the 15 joint specimens taken from rheumatoid arthritis patients who had never received penicillamine.

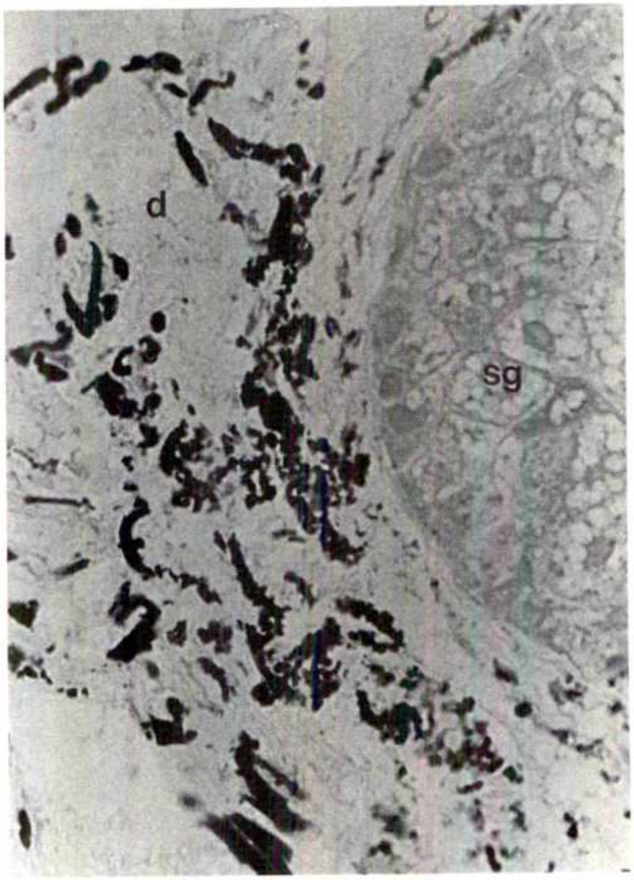

FIGURE 2. 'Bramble-bush' appearance of dermal elastic fibres adjacent to a sebaceous gland in biopsy from pseudo-pseudoxanthomatous skin (orcein). d, dermis; sg, sebaceous gland.

TABLE 2. Details of joint specimens examined for penicillamine-induced elastosis

Patient	Joint	Duration of treatment (years)	Penicillamine-induced elastosis in joint capsule
4	MCP	0	Absent
	Elbow	1	Present
	Shoulder	12	Present
5	Shoulder	7	Present
6	Radial head	9	Present
10	Hip	5	Absent
	Knee	9	Absent
12	Excision ulnar	10	Present
17	Hip	6	Absent
22	Elbow	1	Present
	Knee	5	Absent

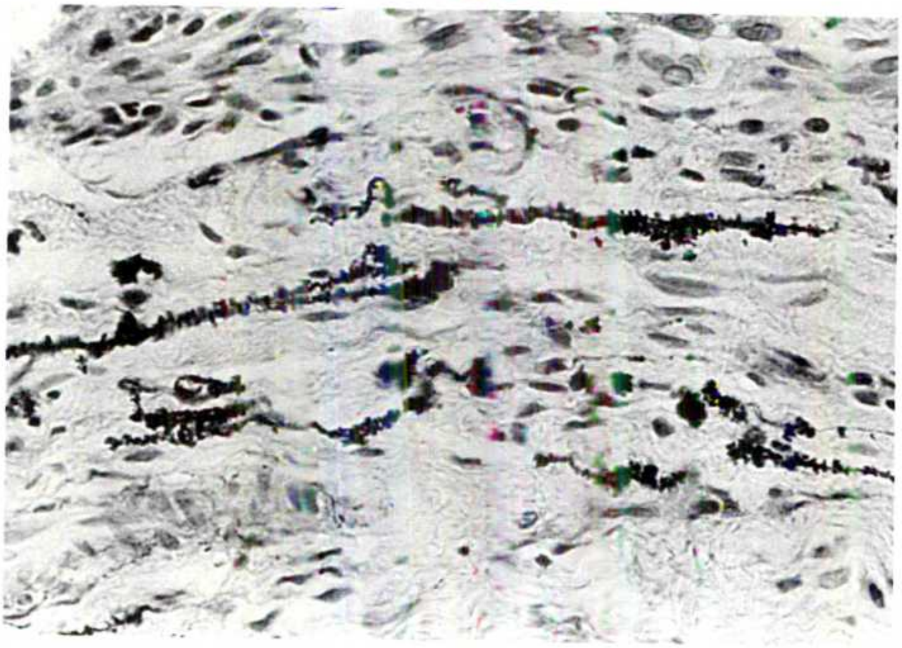

FIGURE 3. Penicillamine-induced elastic fibre damage in joint capsule tissue (orcein).

DISCUSSION

The heavy metal chelator, D-penicillamine, may cause a range of cutaneous problems. Autoimmune bullous skin diseases such as pemphigus vulgaris, pemphigus foliaceous, pemphigus erythematosus or mucous membrane pemphigoid may be induced by penicillamine and in a small number of patients penicillamine has been implicated in the pathogenesis of dermatomyositis.[9] Treatment with penicillamine impairs the maturation and reduces the stability of both collagen and elastin.[10,11] This may contribute to the skin thinning, friability and frictional blistering which may be seen in patients treated with the drug.[9]

The precise mechanism by which penicillamine induces elastic fibre damage is not known. Penicillamine interferes with the formation of stable cross-links in collagen and elastic fibres, both by forming complexes with the cross-linked precursors and by inhibiting lysyl oxidase, the enzyme which catalyses cross-link formation.[11,12] Lysyl oxidase is a copper-dependent enzyme but although penicillamine chelates copper it probably does not inhibit the enzyme by copper depletion.[12] Another essential trace metal may be involved.

Until recently, it was believed that the lower doses of penicillamine used to treat rheumatoid arthritis did not induce elastic fibre damage. This study demonstrates that penicillamine-induced elastic fibre damage does occur in patients with rheumatoid arthritis receiving 'low-dose' penicillamine therapy, and that the damage is not restricted to cutaneous elastic tissue. Penicillamine elastosis was demonstrated in joint capsules after as little as 1 year of treatment and was found in 62% of eight patients or 64% of 11 joints examined. Elastosis in joint tissue usually occurs in the absence of cutaneous elastosis. The clinical significance of this damage is uncertain, but the changes may be focal and were not detected in all specimens from affected individuals. Similarly, elastic fibre damage in patients with high dose penicillamine-induced elastosis is not restricted to the skin but may be associated with more widespread elastic tissue damage in blood vessels and other tissues.[7]

Penicillamine-induced elastic fibre damage was not found in normal skin and none of the patients with rheumatoid arthritis had the gross cutaneous signs which are seen in association with high-dose penicillamine treatment in patients with Wilson's disease or cystinuria. One female patient had subtle pseudoxanthoma elasticum-like changes which were similar to although less marked than those in our previous case.[8] In both these patients the changes were detected on the skin of the neck, but there were no abnormalities in other flexures. Calcification was not present in the involved skin and Bruch's membrane was normal. Damage to the elastic tissue in this membrane, which underlies the retina, causes angioid streaks in hereditary pseudoxanthoma elasticum.

Since our study was completed, EPS has been described in a patient with rheumatoid arthritis taking penicillamine.[13] However, this case had some unusual features. The patient, a 9-year-old child with juvenile rheumatoid arthritis, was prescribed penicillamine, 0·125–0·5 g daily. The drug was stopped after 9 months because of a lack of clinical response when she had received a total dose of approximately 71 g of D-penicillamine. EPS was diagnosed shortly after treatment ceased and the eruption progressed over the following year. Histologically there was calcification in the abnormal elastic fibres. Calcification has not previously been described penicillamine elastosis, but is present in the abnormal elastic tissue of the hereditary form of pseudoxanthoma elasticum. This feature led the authors to suggest that their case may be an unusual variant of EPS or an overlap with hereditary pseudoxanthoma elasticum.[13]

We have demonstrated that penicillamine-induced elastic fibre damage may develop in the skin of patients with rheumatoid arthritis as well as in the capsular tissue of damaged rheumatoid joints. Elastosis was detected after as little as 1 year of relatively low-dose therapy. The reasons for individual susceptibility, the factors leading to localization of penicillamine-induced elastic fibre damage and the significance of the change in terms of tissue function are uncertain.

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Invasive migration of epidemic Kaposi's sarcoma cells *in vitro*

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SUMMARY

Kaposi's sarcoma (KS) is a low grade malignant neoplasm which shows invasive growth and often occurs in immunosuppressed patients with the Acquired Immune Deficiency Syndrome (AIDS; epidemic KS). It is also found in elderly men where it is usually limited to the skin (classic KS). The present study investigated the chemotaxis and invasive migration of epidemic KS cells *in vitro* and compared them to cells grown from classic KS lesions and to fibroblasts. Epidemic KS cells demonstrated invasive migration through reconstituted basement membrane (Matrigel) as well as through interstitial connective tissue (collagen I) in early passages, whereas fibroblasts did not invade either barrier. Epidemic KS cells in late passages did not show any invasive migration. Following pretreatment with tumour necrosis factor α (TNF-α) there was no enhanced migration through the Matrigel and collagen I for epidemic KS cells, whereas classic KS cells showed an increased migration through the type I collagen barrier.

Kaposi's sarcoma (KS) is the most common malignancy in patients with the Acquired Immune Deficiency Syndrome (AIDS), while classic Kaposi's sarcoma is most often circumscribed and affects the elderly. KS in patients with AIDS is widespread and may involve visceral organs as well as the lymph nodes.[1]

Histological studies show that lesions of classic and epidemic (AIDS-associated) KS are composed of endothelial and spindle-shaped cells. Inflammatory cells are present in early lesions.[1-3] The spindle cells show a positive staining with several endothelial cell markers (Factor VIII and BMA 120) and are often considered to be of endothelial origin.[4] Electron microscopic studies have revealed Weibel–Palade bodies indicating the blood vessel origin of these cells in KS.[4]

Recently we established cell cultures from epidemic and classic KS lesions.[5-7] These cells formed no colonies in soft agar and revealed no tumourigenicity in nude mice, but could be

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passaged up to nine times in 0·5% fetal calf serum, whereas cell cultures of human fibroblasts could grow through only 2–3 passages under these conditions. These cells were more than 80% positive with the endothelial detecting agent acetyl-LDL.

The interaction of tumour cells with the extracellular matrix is an important step for invasive growth and the formation of metastases. It is likely that penetration of tumour cells into the basement membrane represents the most crucial step for dissemination of cells and involves distinct events such as attachment to the membrane, secretion of proteolytic enzymes and migration of the tumour cells.[8]

The aim of the present study was to investigate further the nature of cultured KS cells by evaluating their invasive potential through a reconstituted basement membrane and interstitial connective tissue barrier following a chemotactic response to different chemoattractants.

METHODS

Cell culture

Cell cultures were derived from three male HIV positive patients with epidemic KS and from two HIV negative patients with classic KS. Biopsies were explanted or cells were liberated by collagenase digestion as previously described.[5] Fibroblasts were grown from the uninvolved skin of the same patient and were used in the same passage as the KS cells.

Chemoattractants

Fibroblast-conditioned medium (CM) was prepared from confluent monolayers of human fibroblasts. The cells were washed twice with phosphate-buffered saline (PBS) and incubated with serum-free Dulbecco's modified Eagle's medium (10 ml/75 cm^2 flask) for 24 h. The medium was collected, centrifuged to remove cell debris and stored at −20°C.[9] It was used undiluted as a chemoattractant for KS cells and fibroblasts.

Fibronectin (FN) purified from human plasma by chromatography on heparin and gelatin sepharose was provided by Dr M. Aumailley, Max-Planck-Institut für Biochemie, Martinsried, F.R.G. Recombinant platelet-derived growth factor (r-PDGF) was obtained from Amersham. Recombinant human tumour necrosis factor α (TNF-α) was provided by Dr A. Möller, BASF, Ludwigshafen, F.R.G. The specific activity was 3·2 U/mg protein as determined in a 48 h cytotoxic test with fibroblast cell strain L929. The endotoxin contamination of the preparation was < 1·3 pg/ng protein as detected in a Limulus amoebocyte lysate assay. The purity of TNF-α was > 99% as examined in a SDS PAGE electrophoresis.

Confluent layers of epidemic and classic KS cells were incubated with various concentrations of TNF-α (1 : 10 : 100 ng/ml) for 24 h in DMEM with 10% fetal calf serum (FCS). During incubation of the cells in the Boyden chamber the lower and upper compartment contained the same concentration of TNF-α.

Chemotaxis assay

Chemotaxis was assayed using the Boyden blind well chambers with polycarbonate filters (13 mm diameter, 8 μm pore size: Nuclepore Corp., Pleasanton, CA, U.S.A.).[9] These filters divided the chamber into a lower compartment containing the chemoattractant (206 μl of CM, fibronectin [1 μg/ml] or PDGF [0·2 U/ml]) and an upper compartment containing the cell suspension (750 μl 3·0 × 10^5 cells/ml) in serum-free DMEM. The chambers were incubated at 37°C for 4 h. At the end of this period the filters were removed. Cells on the upper side of the filter were scraped off, whereas cells which had migrated to the lower side were fixed in methanol and stained with haematoxylin and eosin. The number of migrated cells was calculated from four unit fields using microscopy at a magnification of × 200. Each experiment was assayed two or three times and run in triplicate.

Invasion assay

To test the invasive migration of normal fibroblasts and classic or epidemic KS cells through different barriers (basement membrane-like (Matrigel) and interstitial connective tissue-like (collagen type I)) we used a modification of a method previously described by Albini *et al.*[10]

The assay was performed in the Boyden chamber containing the chemoattractant in the lower and the cells in the upper compartment. The separating filters were coated with either collagen I gel (225 μl of a 10 mg/ml collagen type I solution with 650 μl of 1·5-fold concentrated DMEM; 173 μg/filter) or a reconstituted basement membrane (Matrigel, 210 μg/filter composed of extracted proteins from the EHS tumour diluted with cold water (Collaborative Research Incorporated, Two Oak Park Bedford, MA 01730, U.S.A.)) containing collagen type IV, heparan sulphate proteoglycan, laminin and nidogin. The gels were then allowed to polymerize for 1 h, at 37°C and the cells were seeded on the top ($3{\cdot}0 \times 10^5$ cells/ml) of the barriers. In order to monitor chemotaxis, the filters were coated with gelatin in a parallel set of experiments. The chambers were incubated and the numbers of migrated cells determined.

Statistical analysis

Data represent the mean values (± SEM) of 18 or 24 estimations. In each individual experiment the statistical significance was assessed by student's *t*-test vs. base line with no TNF-α-treated cells, vs. low/high passage number or vs. CM.

RESULTS

Five cell strains were established from KS tissue from three patients with AIDS and two cell strains from two patients with classic KS (HIV negative). All cell strains were stained with endothelial detecting agent acetyl-LDL and the cells were more than 80% positive. Fibroblasts from biopsies of normal looking skin from the same patients were negative (data not shown).

Epidemic KS cells and fibroblasts as controls were tested for their ability to migrate through a barrier of basement membrane proteins and collagen type I coated onto the porous filters of Boyden chambers. For these studies CM, fibronectin and PDGF were used as chemoattractants. In a parallel set of experiments we monitored chemotaxis of these cells to the same panel of chemoattractants as for the invasion assay.

Epidemic KS cells and fibroblasts showed a positive chemotactic response to all the chemoattractants investigated. Chemotaxis was greatest for CM and fibronectin and to a lesser extent for PDGF (Table 1).

The Matrigel and collagen I barrier reduced the migration of normal fibroblasts and classic KS cells independently of the chemoattractant used (Tables 1 and 2). In contrast, a considerable number of epidemic KS cells migrated through both barriers and this was again independent of the chemoattractant (Table 1). These results were obtained with the early passages (2–4) of epidemic KS cells and fibroblasts. However, when cells in later passages (10–12) were investigated the invasive potential of KS cells was significantly reduced and after the twelfth passage no difference between the epidemic KS cells and fibroblasts was noted (Table 3). The KS cells were also no longer positive for acetyl-LDL uptake. With passages (19–21) of fibroblasts and epidemic KS cells, their chemotactic responses were lost (data not shown).

TABLE 1. Invasive migration and chemotaxis of human skin fibroblasts and epidemic KS cells

Cell type	Attractant	Invasion assay		Chemotaxis assay
		Matrigel	Collagen I	
Fibroblasts	CM	6 ± 4	9 ± 3	75 ± 7
	FN	7 ± 4	12 ± 3	75 ± 5
	PDGF	3 ± 3	11 ± 4	33 ± 4
Epidemic KS	CM	24 ± 4	26 ± 4	74 ± 7
	FN	35 ± 4*	20 ± 4	76 ± 6
	PDGF	21 ± 4	24 ± 5	42 ± 5

* Significant ($P < 0{\cdot}01$) difference compared to CM.

TABLE 2. Invasiveness and chemotaxis of epidemic KS cells and classic KS cells after preincubation with TNF-α

Cell type	TNF-α concentration (ng/ml)	Invasion assay		Chemotaxis assay
		Matrigel	Collagen I	
Epidemic KS	0	23 ± 6	19 ± 6	58 ± 6
	1	17 ± 5	19 ± 5	58 ± 7
	10^1	19 ± 7	22 ± 5	53 ± 6
	10^2	22 ± 5	20 ± 6	57 ± 8
Classic KS	0	9 ± 4	15 ± 5	58 ± 7
	1	13 ± 4*	27 ± 7*	66 ± 7
	10^1	16 ± 5*	25 ± 5*	67 ± 9
	10^2	17 ± 5*	28 ± 6*	61 ± 7

* Significant ($P < 0{\cdot}01$) difference compared to controls (0 TNF).

TABLE 3. Passage dependence of invasive migration and chemotaxis of human skin fibroblasts and epidemic KS cells

Cell type	Passage	Invasion assay		Chemotaxis assay
		Matrigel	Collagen I	
Fibroblasts	2–4	4 ± 4	9 ± 3	67 ± 6
	10–12	7 ± 5	13 ± 4	66 ± 5
Epidemic KS	2–4	23 ± 5*	24 ± 5*	68 ± 6
	10–12	7 ± 5	13 ± 4	61 ± 6

* Significant ($P < 0{\cdot}01$) difference compared to late passage (10–12).

Confluent epidemic and classic KS cells were incubated with TNF-α in concentrations between 1–10^2 ng/ml prior to the assay for 24 h. During the incubation time in the Boyden chamber TNF-α was added in the same concentration as in the preincubation time to the lower and upper compartment of the Boyden chamber.

Two cell strains of classic KS and two cell strains of epidemic KS cells were investigated using CM as a chemoattractant. TNF-α-treated epidemic KS cells showed neither enhanced nor reduced invasive migration through the Matrigel or collagen I barrier and TNF-α had no influence on the CM-induced chemotaxis (Table 2). However, classic KS cells showed a significantly enhanced invasive migration through the collagen I and the Matrigel in comparison to the untreated cells, which was greatest at 100 ng/ml TNF-α (Table 2).

DISCUSSION

Kaposi's sarcoma (KS) is a low grade malignant tumour which differs from other malignant tumours in that it may regress. The chemotactic response of epidemic KS cells to PDGF, CM and FN were investigated and these cells and fibroblasts responded in a similar manner to all the chemoattractants. KS cells showed a response to PDGF which is normally a chemoattractant for fibroblasts and smooth muscle cells but not for endothelial cells. Similar results were reported from Albini *et al.*[11]

The degree of malignancy of classic and epidemic KS cells was analysed by their growth in soft agar and in nude mice and *in vitro* with low serum conditions. They formed no colonies or tumours, but showed an increased number of passages with low concentrations of serum. This indicated a low-grade malignant behaviour for these cells.[3]

To characterize these cells further, we investigated their ability to invade the basement membrane barrier and a reconstituted interstitial connective tissue formed by collagen I. Using CM, fibronectin and PDGF as chemoattractants we found a strong invasive migration through the barriers of the extracellular matrix indicating the malignant properties of epidemic KS cells. Classic KS cells migrated only through collagen I and the number of migrating cells was lower than in the epidemic KS cells. The malignant behaviour was enhanced by treatment of the cells with TNF-α. Fibroblasts from the same patient and in the same passage as the KS cells, used as controls, did not invade the barriers. The results for epidemic KS cells were in good agreement with those of Albini *et al.* who found that epidemic KS cells migrated through Matrigel.[11]

Epidemic KS cells lost their ability to invasively migrate with increased number of passages in culture. In addition, the percentage of cells demonstrating a positive uptake of acetyl-LDL was reduced.[5] This could indicate a modulation of the state of differentiation of the tumour cells due to the culture conditions or the selection of a certain population of cells.

An induction of collagenase by PDGF was previously reported[12] and this could result in an enhanced invasive migration through the collagen I barrier. However, in our experiments, PDGF had no influence on the invasive migration of epidemic KS cells. While fibroblasts showed an enhanced migration towards PDGF, epidemic KS cells did not respond to PDGF.[12,14]

The expression of collagenase in human skin fibroblasts is also induced by TNF-α[13] and fibroblasts have been shown to invade the collagen I after treatment with TNF-α.[14] However, there was no enhanced invasive migration when Matrigel was used as a barrier. A similar response was noted for classic KS cells, whereas epidemic KS did not show any response.

Although the origin of KS cells remains unclear, the data presented here provide strong evidence that epidemic and classic KS cells differ considerably and that both can be differentiated from control fibroblasts.

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Neutrophil zinc levels in psoriasis and seborrhoeic dermatitis

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SUMMARY

The median zinc content of neutrophils was significantly reduced in 16 patients with psoriasis in comparison to both normal controls and six patients with seborrhoeic dermatitis ($P < 0.05$). This reduction was unrelated to the extent of skin involvement. Plasma and erythrocyte zinc levels were unchanged.

Although the role of zinc in psoriasis is uncertain, zinc deficiency in both animals and man is frequently associated with skin lesions. In zinc deficient pigs plaque formation and parakeratosis are readily reversed by zinc supplementation[1] and in man moderate zinc deficiency is often accompanied by an erythematosquamous eruption.[2] The characteristic changes of acrodermatitis enteropathica are seen both in children with severe hereditary zinc deficiency[3] and in some adults on total parenteral nutrition.[4]

Measurements of plasma zinc levels in psoriatic patients have previously produced conflicting results,[5–7] but plasma zinc is an unreliable indicator of tissue zinc levels as it is influenced by many variables that are independent of zinc status.[8] The best available index of tissue zinc in man is probably the level in circulating neutrophils (polymorphonuclear leucocytes, PMN)[9] and this correlates well with one of the major body depots, muscle zinc.[10] In this study, neutrophil zinc levels were studied in patients with psoriasis. Patients with seborrhoeic dermatitis were also studied as this disorder shares many clinical features with psoriasis, but is likely to have a different pathogenesis.

PATIENTS AND METHODS

Patients

Sixteen patients with chronic plaque psoriasis (10 male, six female; age range 21–75 years, mean 37·9 years) and six patients with seborrhoeic dermatitis (six male; age range 27–66 years, mean

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37·6 years) were studied. Patients who had received oral or topical preparations containing zinc within the last 3 months were excluded, as were those with an intercurrent illness or who were receiving regular medication including oral contraceptives. The controls were healthy volunteers and patients with benign skin tumours, and were allocated to one of three groups, matched in age and sex to the patient groups of mild and severe psoriasis and seborrhoeic dermatitis.

The extent of cutaneous involvement was estimated using the 'rule of nines' and classified as severe if the area involved exceeded 18% of total body surface area.

Methods

Venous blood (20 ml) was collected, non-fasting, between 09.30 and 11.30 into acid-washed polystyrene tubes containing preservative-free heparin to give 5 U/ml blood. Whole blood was layered onto a Ficoll–Hypaque gradient and neutrophils were isolated according to the method of Ferrante and Thong.[11] The cells were washed three times in Hanks' balanced salt solution and the few contaminating erythrocytes removed by osmotic lysis. An aliquot of suspension was removed for counting in an haemocytometer and the remainder diluted in 0·3 M nitric acid to give approximately 10^7 cells/ml. Neutrophils accounted for 90–98% (mean 96%) of total white cells counted and platelet contamination was minimal. Plasma was diluted 1 : 5, and washed red cells 1 : 40 in 0·3 M nitric acid. After 1 week the samples were clarified by centrifugation at 400 ***g***, and the zinc concentration of the supernatant measured by flame atomic absorption spectrophotometry (Thermoelectron 257). Calibration standards were prepared from a certified reference solution ('Spectrosol', BDH, Poole, Dorset, U.K.) and the accuracy of the analyses was verified using certified biological reference standards ('Seronorm', Nyegaard, Oslo, Norway). All glassware was acid washed and rinsed twice in deionized water.

The study was approved by the hospital ethical committee and all subjects gave verbal informed consent. The results are expressed as medians with ranges and analysed using the Mann–Whitney test.

RESULTS

The neutrophil and plasma zinc concentrations are shown in Tables 1 and 2, respectively, and graphically in Figures 1 and 2, respectively. Cell clumping precluded determination of neutrophil zinc levels in two patients with mild psoriasis. The median neutrophil zinc values were significantly lower in the psoriasis group than in their matched controls ($P < 0{\cdot}05$, Mann–Whitney test). There was no statistical difference between the median neutrophil zinc level of the three control groups, nor was there a difference between the levels of neutrophil zinc of patients with mild and severe psoriasis (Zn 66·7 μg/10^{10} vs. 70·0 μg/10^{10} cells, respectively; 1 μg zinc = 15·38 nmol).

In contrast to patients with psoriasis, those with seborrhoeic dermatitis showed no evidence of neutrophil zinc depletion. None of the groups significantly differed in plasma or red blood cell zinc levels, although there was a tendency for those with severe psoriasis to have slightly higher erythrocyte zinc levels (Table 3).

DISCUSSION

Depletion of neutrophil zinc in patients with psoriasis may be specific to this disorder since zinc levels were normal in patients with seborrhoeic dermatitis. Wasik *et al.*[12] have previously found

TABLE 1. Neutrophil zinc levels in patients with psoriasis and seborrhoeic dermatitis

Neutrophil zinc levels: (μg/10^{10} cells)					
Mild psoriasis		Severe psoriasis		Seborrhoeic dermatitis	
Patients	Matched controls	Patients	Matched controls	Patients	Matched controls
64·3	72·1	70·0	98·7	100·0	69·5
66·7	95·0	54·4	77·0	66·4	95·0
70·2	84·2	73·2	62·4	90·0	86·5
69·0	59·0	78·1	71·0	88·9	77·0
64·5	69·5	73·3	86·5	97·7	72·1
77·1	80·3	54·4	79·7	100·0	96·9
64·3	120·8	42·2	78·4	—	—

TABLE 2. Plasma zinc levels in patients with psoriasis and seborrhoeic dermatitis

Plasma zinc levels: (μg/ml)					
Mild psoriasis		Severe psoriasis		Seborrhoeic dermatitis	
Patients	Matched controls	Patients	Matched controls	Patients	Matched controls
0·55	0·70	0·86	0·72	0·92	0·89
0·84	0·94	0·89	0·61	0·97	0·94
0·67	0·66	0·67	0·86	0·90	0·72
0·87	0·72	0·64	0·65	0·81	0·61
0·87	0·89	0·74	0·76	0·67	0·70
0·72	0·90	0·89	0·90	0·65	1·05
0·96	0·65	0·63	0·72	—	—
0·95	0·71	—	—	—	—
0·89	0·90	—	—	—	—

a reduction in total leucocyte zinc in patients with both active and quiescent psoriasis, but this is a less specific marker of zinc status.[13] The lack of any difference in erythrocyte zinc levels may imply that there is no long-term severe zinc depletion, although erythrocyte zinc is of dubious value in the assessment of zinc stores.[8]

The epidermis is rich in zinc,[14] and there are several mechanisms by which zinc depletion might be related to the pathogenesis of skin disease.[15-18] The finding of reduced zinc levels in neutrophils is intriguing as infiltration of the epidermis by neutrophils and the formation of microabscesses in the stratum corneum are major features of psoriasis.[19]

If reduced neutrophil zinc levels are indicative of generalized tissue zinc depletion in

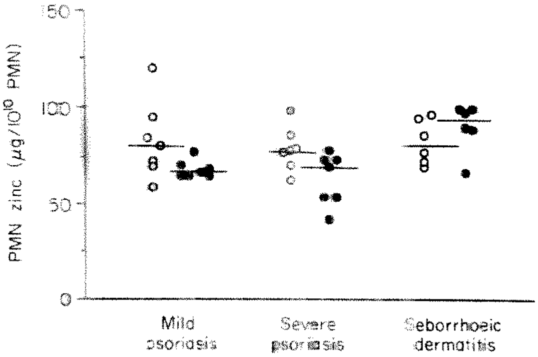

FIGURE 1. Neutrophil zinc levels in controls and patients with psoriasis and seborrhoeic dermatitis (○ controls, ● patients, bars indicate median levels).

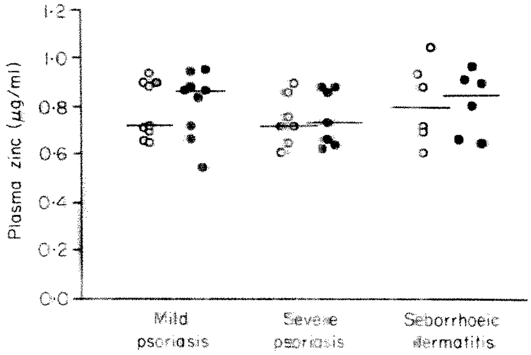

FIGURE 2. Plasma zinc levels in controls and patients with psoriasis and seborrhoeic dermatitis (○ controls, ● patients, bars indicate median levels).

TABLE 3. Red blood cell zinc (μg/g Hb) in patients and controls

	All psoriasis		Mild psoriasis		Severe psoriasis		Seborrhoeic dermatitis	
	Control	Patients	Control	Patients	Control	Patients	Control	Patients
n	14	14	7	7	7	7	6	6
Median	35·1	36·7	37·0	33·8	33·8	40·9	32·7	36·5
Range	20·9–46·0	30·7–52·6	28·3–44·7	30·7–43·5	25·9–46·0	36·7–52·6	28·3–44·7	30·6–40·6

All differences non-significant.

psoriasis, the cause of this is unclear. Malabsorption has been associated with severe skin disease,[20] but we found no other evidence of this in our patients on routine biochemical and haematological testing; and neutrophil zinc depletion was independent of the extent of the disease. Depletion might be precipitated by excessive zinc loss due to desquamation, particularly as many diets are marginal in zinc. However, such losses are small in comparison to faecal and urine zinc excretion[21] and can probably be compensated for by normal homoeostatic mechanisms. Our results, rather than indicating zinc depletion, may reflect a movement of zinc from neutrophils to another tissue compartment, stimulated perhaps by circulating inflammatory mediators. A further trial of oral zinc therapy with monitoring of neutrophil zinc levels and clinical assessment of the disease is indicated.

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Autologous mixed lymphocyte reaction is reduced in patients with psoriasis

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SUMMARY

The autologous mixed lymphocyte reaction (auto-MLR) was studied to test the interactions between immunocompetent cells in patients with psoriasis. The auto-MLR in 20 patients with psoriasis was significantly lower than in 16 normal controls. Lower values were found in untreated psoriatic patients than in those in remission following treatment. The values in the latter group were significantly lower than in controls and in six patients with atopic dermatitis in remission. The tendency for an increase in the auto-MLR with a decrease in disease activity was further confirmed in five patients studied before and after treatment. In contrast, the allogeneic lymphocyte reaction (allo-MLR) in psoriatics was similar to that in normal controls.

In psoriasis the mononuclear cells are a feature of the inflammatory cell infiltrate. It is likely that cell-mediated immune mechanisms play an important role in the development of the characteristic neutrophil cell migration and the epidermal cell proliferation seen in this disorder.[1] There is accumulating evidence that cellular immune mechanisms are involved in the pathogenesis of psoriasis,[2–4] and the dramatic improvement seen with cyclosporin treatment, suggests that immune cellular mechanisms are involved.[4–6]

The autologous mixed lymphocyte reaction (auto-MLR) is an immunological response by T cells to surface antigens on autologous non-T cells such as B lymphocytes, macrophages and dendritic cells.[7] Class II MHC antigens are important in stimulating this response.[8] The auto-MLR is altered in those diseases considered to be mediated by the immune system, such as systemic lupus erythematosus,[9] rheumatoid arthritis[10] and atopic dermatitis.[11,12] No alteration of the auto-MLR has been reported in psoriasis, though a significant increase in autologous mixed-epidermal-cell-lymphocyte reaction involving the *in vitro* stimulation of T cells by isolated epidermal cells from the lesion has been found.[13] However, in contrast Faure *et al.*[14] found no such increase.

Recently, it has been reported that the use of fetal calf serum (FCS) in the culture medium affected the results of the auto-MLR, the FCS acting as a xenogenic protein to produce

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interleukin (IL) 2 and to induce suppressor/cytotoxic T cells.[15] The presence of FCS in previous studies has modulated the results of the immune reaction *in vitro*.

We have assayed the auto-MLR in psoriasis with 20% autologous serum and using nylon-wool-adherent stimulator cells and non-adherent responder cells that were separated only in the presence of the serum. We have also studied the allogeneic mixed-lymphocyte reaction (allo-MLR) under the same experimental conditions.

METHODS

Preparation of responder and stimulator cells in auto-MLR

RPMI 1640 medium (Gibco, Grand Island, NJ, U.S.A.) was used throughout the experiments and contained L-glutamine (Sigma, St Louis, MO, U.S.A.) and 5×10^{-5} M 2-mercaptoethanol, which was supplemented with 20% heat-inactivated fresh autologous human serum (AHS).

Venous blood (80 ml) was taken from each of 20 patients with psoriasis (age 43 ± 13 years), from six patients (20 ± 3 years) with atopic dermatitis in remission, and 16 normal volunteers (30 ± 5 years). Of the 20 patients with psoriasis, nine were in remission after treatment with various therapies that included PUVA (three patients), Goeckerman regime (five patients), oral etretinate (one patient) and 11 were on no treatment. The mononuclear cells were isolated from the venous blood by Ficoll–Isopaque gradient centrifugation,[16] and AHS was used instead of FCS. The separated mononuclear cells were washed three times and then suspended in the RPMI/AHS medium.

The mononuclear cells ($1–3 \times 10^8$) in 2 ml of RPMI/AHS medium were added drop by drop to a 20 ml glass syringe that had been packed with 5 g of nylon wool (Nylon Fiber, Wako Pure Medical Company, Osaka, Japan) and then drained with RPMI/AHS equilibrated at 37°C in an incubator for 60 min. The column was then washed with an additional 1 ml of warm RMPI/AHS medium at a flow rate of one drop per s, and the non-adherent cells were used as the responder T cells. To obtain the stimulator cells, nylon-wool-adherent cells were recovered by transferring the nylon wool to a Petri dish containing RPMI 1640 medium and teasing it apart with forceps. The cells collected were then centrifuged at 200 ***g*** for 10 min and resuspended in the RPMI/AHS medium. The stimulator cells were X-ray irradiated (30 Gy) before the MLR assay.

Preliminary studies performed in some subjects showed that the responder cell fractions contained more than 82% T cells as determined by their reaction with monoclonal antibody and FACS analysis. Less than 0·4% B cells were detected by surface immunoglobulin. The stimulator cell fractions contained about 60% surface Ig^+ B cells, 15–25% monocytes and less than 8% T cells, the rest being non-T and non-B cells.

MLR assays

For the auto-MLR, 1×10^7 T cells plus 1×10^7 stimulator cells were incubated at 37°C in 95% air and 5% CO_2 for 5 days in 24-well flat-bottomed culture plates (Nunc, Roskilde, Denmark) in a total volume of 2 ml. Eighteen hours before harvest, 0·5 μCi of tritiated thymidine (^{3}H-TdR; specific activity 5 Ci/mM; Radiochemicals, Amersham, U.K.) was added to each well, and the amount of uptake measured.

For the allo-MLR assay, the responder cells from a donor and X-ray-treated stimulator cells from another allogenic donor were used. Fresh autologous serum from the responder was used for the cell separation medium.

The data were expressed as the mean cpm and standard deviation of triplicate cultures. The significance of differences between the means of the various groups and in the group of patients before and after successful treatment was determined using the unpaired Student's *t*-test and paired *t*-test, respectively.

RESULTS

The mean value of auto-MLR in patients with psoriasis was 4115 ± 3784 cpm and was significantly lower than that of 16 normal controls, 30,989 ± 16,336 cpm ($P < 0{\cdot}001$) as shown in Figure 1.

Comparison of the results for auto-MLR in patients with active psoriasis with those who were in remission with treatment showed that the auto-MLR was significantly higher in those who were in remission ($P < 0{\cdot}001$). However, even the values of the auto-MLR in those patients with psoriasis in remission was still much lower than those of normal controls ($P < 0{\cdot}001$) and were significantly lower than the values obtained from six patients with atopic dermatitis in remission ($P < 0{\cdot}001$) as shown in Figure 2. In contrast, there was no difference between the results of the auto-MLR in normal controls and those who had atopic dermatitis.

In a sequential study performed on five patients with psoriasis, the auto-MLR was found to increase significantly following treatment ($P < 0{\cdot}001$) as shown in Figure 3. There was no difference in the values obtained in those in remission, whatever their treatment.

In contrast, the mean value of the allo-MLR in psoriasis was only slightly lower than in normal controls (Fig. 4). In the induction of the allo-MLR, the psoriatic stimulator cells were as effective as non-psoriatic stimulator cells.

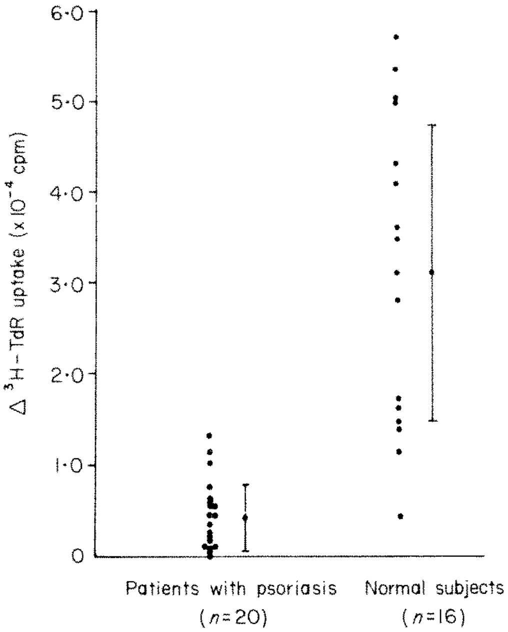

FIGURE 1. Comparison of auto-MLR in patients with psoriasis and normal individuals. Each bar represents the mean and standard deviation.

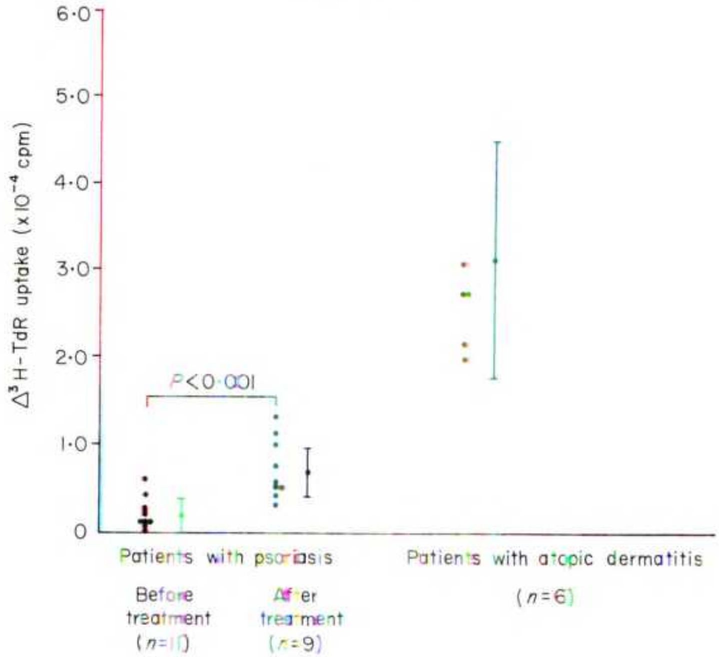

FIGURE 2. Auto-MLR in psoriatic patients with active lesions (before treatment) and in those in remission (after treatment) in comparison with auto-MLR in patients with atopic dermatitis who were in remission after successful treatments. Bars, means ± standard deviations.

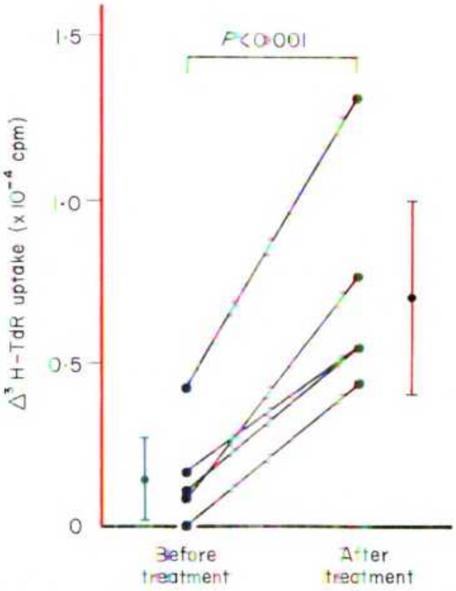

FIGURE 3. Sequential study of auto-MLR in five patients before and after successful treatment of psoriasis. Bars, means ± standard deviations.

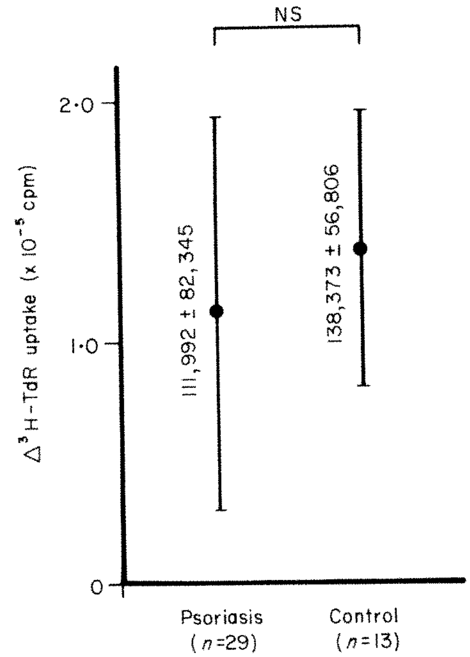

FIGURE 4. Comparison of allo-MLR in patients with psoriasis and controls. Bars, means ± standard deviations. NS, no significant difference.

DISCUSSION

The present study demonstrates that the auto-MLR, in conditions in which the whole procedure for cell separation and culture was carried out in culture medium supplemented with 20% autologous human serum, produced significantly lower active proliferation of responder cells in patients with psoriasis as compared with normal healthy controls. In contrast, the allo-MLR in patients with psoriasis was similar to that of normal individuals, excluding the possibility that either the stimulator cells or the responder cells exerted any suppressor activity.

Our results contrast with those of Schopf *et al.*[13] who reported that the auto-MLR assay in psoriasis was similar to that of healthy controls. It is possible that the different cell separation methods, culture conditions and cell populations used might account for the different findings. We have used far larger numbers of interacting cells, two logarithmic orders higher, and a four times higher concentration of autologous serum in the culture medium. In addition, the ratio of responder T cells to stimulator cells in our study was more than twice theirs.[13]

We found that patients with psoriasis after effective treatment had an elevation of the auto-MLR, but the level was still less than that of normal controls. A reduced auto-MLR is associated with skin inflammation, and has been reported in patients with atopic dermatitis.[11,12] However, in our present study, even in remission, auto-MLR in patients with psoriasis was significantly lower than those with atopic dermatitis. The reduced auto-MLR appears to be confined to the patients with psoriasis and not a phenomenon secondary to extensive skin inflammation.

The effects of serum factors should be taken into consideration because of our use of autologous serum in the culture system. Serum abnormalities that affect neutrophil/lymphocyte functions have been described in psoriasis.[1,2,17-19] We have studied the effects of exchange of serum on the auto-MLR between a patient with active psoriasis and a normal individual. When the autologous serum was replaced in the normal individual with the serum from a 56-year-old patient with psoriasis, the value of the auto-MLR was markedly reduced from 29,633 ± 7124 cpm to 8655 ± 1473 cpm. It has been reported that the serum of patients contained factors that interfered with γ interferon-induced expression of HLA-DR antigen on monocytes,[20] and the reduction of HLA-DR antigen expression on stimulator cells may incur a decrease in MLR. The influence of these factors in the serum is unlikely in our system. The inhibitory activity in the serum of patients would be abolished by the addition of superoxide dismutase, 2-mercaptoethanol and by indomethacin.[20] The reduced auto-MLR in psoriasis cannot be explained simply by the influence of serum factors because a similar exchange experiment with an allogeneic serum from a normal individual was unable to induce any increase in the auto-MLR of the psoriatic patient (6449 ± 1467 cpm–6268 ± 652 cpm). This result, with the findings of reduced levels of auto-MLR in patients with psoriasis even following successful treatment of their skin lesions, suggests that there is an inherent defect in the T-cell response to autologous non-T cells in psoriasis.

In conclusion, although the influence of inhibitory serum factors cannot be ruled out in those with active lesions, there appears to be a defect in the self-recognition mechanism of T lymphocytes in patients with psoriasis. The role of the reduced auto-MLR in the pathogenesis of psoriasis is unknown. One possibility is that it may be related to suppressor dysfunction of an immunoregulatory network as noted in other autoimmune disorders. Recently $T4^+$ $2H4^+$ cells, the suppressor/inducer T-cell population, were found to be triggered maximally in the auto-MLR rather than $T4^+$ $4B4^+$ cells (helper/inducer cells). Perturbation of the $T4^+$ $2H4^+$ cells may lead to profound consequences including autoreactivity and tissue destruction.[21,22] Sauder *et al.* have reported a suppressor cell defect in psoriasis and have studied the ability of lymphocytes to suppress the MLR.[23]

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Differential enhancement of interferon-γ-induced MHC class II expression of HEp-2 cells by 1,25-dihydroxyvitamin D_3

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SUMMARY

Interferon-γ (IFN-γ) induces the expression of MHC class II antigens on non-lymphoid cells. In this study we investigated the effect of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the most active metabolite of vitamin D_3, on the IFN-γ-induced expression of MHC class II antigens on HEp-2 cells. We found that $1,25(OH)_2D_3$ enhanced the IFN-γ-induced expression of HLA-DR on these cells. Differential effects on the MHC class II antigens HLA-DR, -DQ and -DP were also observed.

The expression of MHC class II antigens on non-lymphoid cells is dependent on lymphokines.[1] Interferon-γ (IFN-γ) is a product of activated T lymphocytes and one of the major immunoregulatory lymphokines, one of its functions being the expression of MHC class II antigens on non-lymphoid cells.[2] IFN-γ has been shown to induce MHC class II antigens on keratinocytes,[3] and it has been shown that these keratinocytes can maintain the clonal expansion of already activated lymphocytes, although they were unable to induce the proliferation of resting lymphocytes.[4] These keratinocytes could be a target of MHC class II antigen-specific cytotoxic T lymphocytes (CTL).[5] A recent study has shown that keratinocytes expressing class II antigens induced an antigen-specific unresponsive state in hapten-specific Th1 clones.[6]

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is the most active metabolite of vitamin D_3 and an important factor in calcium homeostasis. Recently, receptors for $1,25(OH)_2D_3$ have been found in tissues and cell lines other than those classically involved in calcium regulation.[7] Since the discovery of these receptors in the mononuclear leucocytes, further data have accumulated suggesting an immunological role for $1,25(OH)_2D_3$.[8,9] However, there have been only a few reports on the effects of $1,25(OH)_2D_3$ on the expression of MHC class II antigens.[10,11] In this study we have investigated the effects of $1,25(OH)_2D_3$ on IFN-γ-induced expression of MHC class II antigens in the human squamous cell carcinoma cell line, HEp-2.

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METHODS

Cell lines and cell culture

The human epithelial cell line (HEp-2) was obtained from Dainippon Pharmaceutical Co Ltd, Osaka, Japan, and was maintained in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum (FCS).

Human IFN-γ and 1,25(OH)$_2$D$_3$

Recombinant IFN-γ was purchased from Collaborating Research Incorporated, Two Oak Park, Bedford, MA, U.S.A. and 1,25(OH)$_2$D$_3$ was a gift from the Chugai Pharmaceutical Co Ltd.

Serological reagents

The following monoclonal antibodies were used: anti-HLA-DR (monomorphic, clone L243), anti-HLA-DQ (Leu 10, clone SK10) and anti-HLA-DP (monomorphic, Clone B7/21) (Becton-Dickinson, Mountain View, CA, U.S.A.). For an isotype-matched control, monoclonal antibody Lyt 1 (Becton-Dickinson) was used. These antibodies were diluted 1:100. A fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (F(ab′)$_2$ fragment) (Becton-Dickinson) was used to visualize the HEp-2 cells in a dilution of 1:20.

Induction of MHC class II antigen on HEp-2 cells

The HEp-2 cells (1×10^5/ml) were plated into each well of a 24-well plate (Corning). These cells were cultured with either IFN-γ or 1,25(OH)$_2$D$_3$ at 37°C in 5% CO$_2$ for 48 or 72 h. The concentrations of IFN-γ were 10, 100 or 1000 IU/ml and those of 1,25(OH)$_2$D$_3$ were 10^{-8} M or 10^{-10} M. These cells were then treated with 0·25% trypsin (Sigma, St Louis, MO, U.S.A.) and then washed three times with RPMI 1640 medium. The cells were then stained for MHC class II antigens.

Immunofluorescence procedure

The HEp-2 cells were incubated with a 1:100 dilution of anti-DR, anti-DQ, anti-DP or with anti-Lyt-1 antibody for 60 min at 4°C and then washed three times with the RPMI 1640 medium. The cells were then incubated with 1:20 diluted F(ab′)$_2$ fragment of FITC-conjugated goat anti-mouse IgG at 4°C for 60 min and then washed three times with the RPMI 1640 medium. The cells were then examined for fluorescence according to previously described methods.[12]

Statistical analysis

The experiments were performed at least four times and at least 400 cells were counted in triplicate, and the results were expressed as arithmetic mean value ± standard deviation. The Student *t*-test was used and a *P* value of less than 0·05 was considered to be significant.

RESULTS

Induction of HLA-DR on HEp-2 cells by IFN-γ

After 48 h incubation with IFN-γ in concentrations of 100 IU/ml or 1000 IU/ml, HLA-DR expression was 1 and 10·4%, respectively. When incubated for 72 h, HLA-DR expression was 16 and 34·5%. HEp-2 cells incubated with 10 IU/ml or without IFN-γ did not show any HLA-DR expression even after 72 h incubation.

TABLE 1. Percentage of HLA-DR positive HEp-2 cells after incubation with IFN-γ and/or $1,25(OH)_2D_3$

IFN-γ (IU/ml)	$1,25(OH)_2D_3$					
	0		10^{-10} M		10^{-8} M	
	48 h	72 h	48 h	72 h	48 h	72 h
0	0	0	0	0	0	0
100	0·6 ± 0·2	16·2 ± 1·4	8·5 ± 1·2*	42·0 ± 4·9*	10·1 ± 0·8*	43·9 ± 4·7*
1000	10·4 ± 2·1	34·5 ± 4·1	17·3 ± 1·7**	47·3 ± 3·1**	27·9 ± 1·5*	65·4 ± 4·1*

Values are means ± SD.
* $P < 0·01$ and ** $P < 0·05$ compared to IFN-γ alone.

$1,25(OH)_2D_3$ enhanced the IFN-γ-induced expression of HLA-DR on HEp-2 cells

Varying doses of IFN-γ (100, 1000 IU/ml) and/or $1,25(OH)_2D_3$ (10^{-8}M, 10^{-10} M) were added to the HEp-2 cells which were incubated for 48 and 72 h. The data are shown in Table 1. The IFN-γ-induced expression of HLA-DR was significantly enhanced by $1,25(OH)_2D_3$ in both a time-dependent and a dose-dependent fashion, although $1,25(OH)_2D_3$ alone did not induce any expression of HLA-DR.

Time of action of $1,25(OH)_2D_3$

$1,25(OH)_2D_3$ only enhanced the IFN-γ-induced expression of HLA-DR when it was present at an early stage of incubation (Fig. 1). When $1,25(OH)_2D_3$ was added after 24 h it did not effect the IFN-γ-induced expression of HLA-DR on the HEp-2 cells.

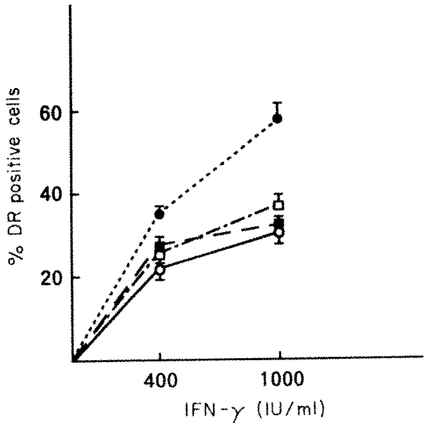

FIGURE 1. $1,25(OH)_2D_3$ was necessary at an early stage of incubation for an enhancement of IFN-γ induced expression of HLA-DR on HEp-2 cells. IFN-γ was added at the beginning of incubation. $1,25(OH)_2D_3$ was added to the well at the beginning of the culture (●- - - -●), 24 h after incubation (□- - - -□) or 48 h after incubation (■- - - -■). As a control, $1,25(OH)_2D_3$ was not added to the well (○- - - -○). HLA-DR positive cells were counted after visualization with indirect immunofluorescence. Bars = SD.

Maintenance of HLA-DR expression of HEp-2 cells after removal of IFN-γ and/or $1,25(OH)_2D_3$

The effect of IFN-γ and/or $1,25(OH)_2D_3$ on HLA-DR expression after their removal from the medium was also studied. After 3 days of culture the medium was changed completely and the HLA-DR expression assessed at days 4, 7 and 10. As shown in Figure 2, enhancement by $1,25(OH)_2D_3$ could no longer be seen on day 4. Expression of HLA-DR by HEp-2 cells was maintained even at day 10, 6 days after the removal of IFN-γ and/or $1,25(OH)_2D_3$. The percentage of positive cells and the intensity of fluorescence however declined gradually.

Differential expression of MHC class II antigens by HEp-2 cells incubated with IFN-γ and/or $1,25(OH)_2D_3$

The effect of IFN-γ and/or $1,25(OH)_2D_3$ was studied on the expression of HLA-DQ and HLA-DP (Table 2). IFN-γ induced the expression of HLA-DP on HEp-2 cells although this was less marked than the expression of HLA-DR. $1,25(OH)_2D_3$ did not induce the expression of HLA-DP on HEp-2 cells but enhanced IFN-γ-induced expression of this class II antigen on HEp-2 cells. IFN-γ and/or $1,25(OH)_2D_3$ did not induce the expression of HLA-DQ, thus a differential enhancement by $1,25(OH)_2D_3$ on the IFN-γ-induced expression of MHC class II antigens was observed.

DISCUSSION

Recent evidence suggests that $1,25(OH)_2D_3$ plays a much wider role in cell biology than that of the regulation of calcium metabolism.[7] It is capable of inhibiting the production of immunoglobulins and the proliferation of B cells,[8] and inhibits the production of IL-2 and the proliferation of T cells[9] and induces the differentiation of myeloid leukaemia cells in the mouse.[13] The modulation of MHC class II antigen expression by $1,25(OH)_2D_3$ has been reported,[10,11] and also that it enhanced the IFN-γ-induced expression of Ia by the murine monocytic Wehi-3 cells, in a dose- and time-dependent fashion.[10] However, it was reported for

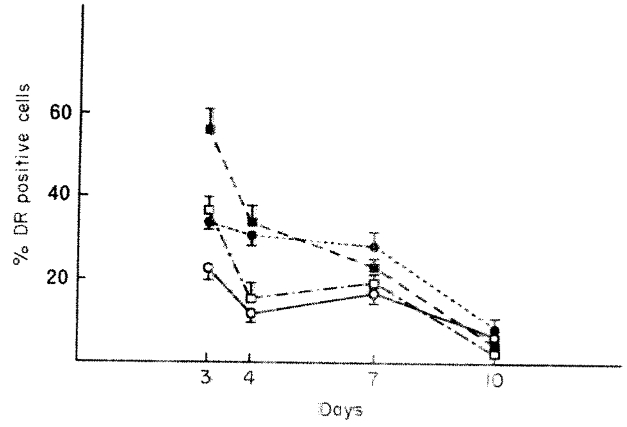

FIGURE 2. Maintenance of HLA-DR expression on HEp-2 cells after removal of IFN-γ and/or $1,25(OH)_2D_3$. After 3 days of culture with IFN-γ and/or $1,25(OH)_2D_3$, the medium was completely changed and the expression of HLA-DR was assessed at days 4, 7 and 10 using indirect immunofluorescence. ○——○, IFN-γ 400 IU/ml, $1,25(OH)_2D_3$ 0; ●- - - -● IFN-γ 1000 IU/ml, $1,25(OH)_2D_3$ 0; □- - - -□ IFN-γ 400 IU/ml, $1,25(OH)_2D_3$ 10^{-8} M; ■- - - -■ IFN-γ 1000 IU/ml, $1,25(OH)_2D_3$ 10^{-8} M. Bars = SD.

TABLE 2. Percentage of HLA-DR, -DP, -DQ positive HEp-2 cells after incubation with IFN-γ and/or $1,25(OH)_2D_3$

	$1,25(OH)_2D_3$								
	0			10^{-10} M			10^{-8} M		
IFN-γ(IU/ml)	HLA-DR	HLA-DP	HLA-DQ	HLA-DR	HLA-DP	HLA-DQ	HLA-DR	HLA-DP	HLA-DQ
0	0	0	0	0	0	0	0	0	0
10	0	0	0	0·9 ± 0·3	0	0	7·9 ± 4·1	0	0
100	25·8 ± 2·2	3·0 ± 2·2	0	38·8 ± 1·9*	1·0 ± 0·2	0	61·0 ± 5·4*	5·0 ± 2·2	0
1000	29·5 ± 0·6	9·1 ± 1·5	0	49·0 ± 2·4*	15·7 ± 2·5**	0	79·7 ± 4·8*	24·3 ± 1·9*	0

Values are means ± SD.
* $P < 0·01$ and ** $P < 0·05$ compared to IFN-γ alone.

the MHC class II antigen-expressing melanoma cell line, Du-Mel 17, that the expression of class II antigens was considerably reduced by $1,25(OH)_2D_3$.[11] The reason for this is not yet known and further studies are indicated.

In this study, we have investigated the effect of $1,25(OH)_2D_3$ on the IFN-γ-induced MHC class II antigen expression on HEp-2 cells, and found that it enhanced this expression. A differential effect on class II antigens HLA-DR, -DQ and -DP was also observed. A similar differential induction of MHC class II antigens by IFN-γ has been reported on dermal fibroblasts[14] and keratinocytes,[15] and our results obtained with HEp-2 cells using immunofluorescence techniques were similar to those obtained with fibroblasts. The differential enhancement of the IFN-γ-induced expression of class II antigens with $1,25(OH)_2D_3$ on HEp-2 cells was observed. Further studies are in progress on the effect of $1,25(OH)_2D_3$ on the expression of MHC class II antigens on human keratinocytes.

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Pentoxifylline inhibits the proliferation of human fibroblasts derived from keloid, scleroderma and morphoea skin and their production of collagen, glycosaminoglycans and fibronectin

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SUMMARY

Pentoxifylline, an analogue of the methylxanthine theobromine, inhibits the proliferation and certain biosynthetic activities of fibroblasts derived from normal human skin. Fibroblasts from the skin of patients with keloids, scleroderma and morphoea were cultured *in vitro* in the presence and absence of pentoxifylline (100–1000 μg/ml) to determine whether it inhibits fibroblast proliferation and the production of collagen, glycosaminoglycans (GAG), fibronectin and collagenase activity. The exposure of subconfluent fibroblast cultures to pentoxifylline resulted in non-lethal, dose-dependent reductions in serum-driven fibroblast proliferation, with 1000 μg/ml pentoxifylline virtually negating the proliferative effect of serum on the cells. The fibroblasts assayed as confluent cultures produced reduced amounts, by up to 95%, of collagen and GAG, dependent on the concentration of pentoxifylline, both in the presence and absence of serum. Pentoxifylline similarly inhibited the fibronectin production by keloid and scleroderma fibroblasts, but had no effect on collagenase activity.

Fibroblasts are the major cell type responsible for maintaining the integrity of the extracellular matrix found in the dermis of the skin and other connective tissues. Under basal conditions, the fibroblasts maintain the extracellular matrix by synthesis and degradation of its components.[1] This balance of synthesis and degradation is only transiently altered during normal repair processes such as occurs in wound healing, and abnormalities in matrix component metabolism may be responsible for the excessive fibrosis that occurs in scleroderma,[2] morphoea,[3] cutaneous

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keloids and hypertrophic scars.[4,5] Fibroblasts derived from these areas of fibrosis produce an excess of extracellular matrix components that includes collagens,[6–9] fibronectin[9] and GAGs.[10]

To develop a therapy for these dermal fibrotic disorders, we have tested several cytokines and drugs on cultures of dermal fibroblasts for their ability to inhibit the synthesis of matrix components and/or to stimulate their degradation.[11–15] One compound, pentoxifylline, an analogue of methylxanthine theobromine, was found to have an anti-fibrotic effect.[16,17] We have recently reported that pentoxifylline treatment of normal dermal fibroblasts results in an inhibition of their proliferation and the production of collagen, GAG and fibronectin with enhancement of collagenase activity.[15] To determine whether pentoxifylline has a similar effect on fibroblasts derived from fibrotic skin, we have studied the effect of this compound on the proliferation of fibroblasts derived from keloids, scleroderma and morphoea and the production by these cells of matrix components.

METHODS

Fibroblast cultures

Primary cultures of fibroblasts were initiated from biopsies of a patient with keloids,[7] from three patients with scleroderma[12] and from inflammatory and fibrotic skin of morphoea, using an explant method. The explants were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM Hepes, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin plus 20% heat-inactivated fetal calf serum (FCS) (Whittaker-MA Bioproducts, Walkersville, MA, U.S.A.) at 37°C in a 5% CO_2 humidified atmosphere. The fibroblast cultures were subcultured by trypsinization and cells from the third passage were then assayed for their proliferative and biosynthetic activities.

Assay of fibroblast growth

The assay of FCS-driven fibroblast growth was performed using asynchronous non-confluent cultures by placing 1500 freshly trypsinized fibroblasts in 200 μl of DMEM-10% FCS into triplicate 0·45 cm^2 microculture wells (Microtest III, Falcon Labware, Oxnard, CA, U.S.A.) and incubating for 18 h at 37°C in a 5% CO_2 atmosphere. After adherence of the cells to the well bottoms, the medium was removed and replaced with 200 μl fresh DMEM ± 20% FCS with or without different concentrations of pentoxifylline (in the DMEM). The cultures were incubated for an additional 96 h, and afterwards the fibroblasts were trypsinized and the number of cells counted in a haemocytometer, and their viability assessed with trypan blue exclusion. The assays were performed using triplicate cultures and each culture was counted twice and the values expressed as the number of cells per microwell ± standard deviation (SD).

Assay of fibroblast collagen production

The assay of collagen production during a 24 h period by the confluent fibroblasts cultured in the presence of ascorbic acid with and without 10% FCS was assessed by the incorporation of ^{3}H-proline into the collagenous protein. The use of confluent cultures minimizes growth-related events. The freshly trypsinized fibroblasts were plated in triplicate in 0·45 cm^2 microcultures at a near confluent density with 25,000 fibroblasts per well in 200 μl DMEM-10% FCS and incubated for 48 h to produce a totally confluent monolayer of cells. The medium was then removed and replaced with 200 μl DMEM alone or DMEM ± 10% FCS containing 50 μg/ml ascorbic acid with and without pentoxifylline and incubated for a further 48 h. The cultures were then pulsed with 0·5 μCi of ^{3}H-proline (31 Ci/mmol; Amersham Corp, Arlington Heights, IL, U.S.A.) and 50 μg/ml β-aminopropionitrile for the final 24 h of culture. The incorporation of ^{3}H-proline into the pepsin-resistant, salt-precipitated extracellular collagen was then determined as has been previously described.[11,12,14,15] Each experiment was performed in triplicate wells and the average result expressed as disintegrations per min (dpm) of ^{3}H-collagen per 10^3 cells ± SD, with the cell numbers determined by haemocytometer counting the numbers of trypsin-released cells in a series of identically treated microcultures.

Assay of fibroblast glycosaminoglycan (GAG) production

Confluent microcultures treated identically to those described for the assay of collagen production were used to assess the GAG production. These cultures were incubated without β-aminopropionitrile and labelled with 0·5 μCi of ^{3}H-glucosamine (22 Ci/mmol, Amersham) for the last 24 h of culture. The incorporation of ^{3}H-glucosamine into cetylpyridinium chloride-precipitated GAG in the culture medium and cell layer was then determined as previously described.[13-15] Each experiment was performed in triplicate wells and the average results expressed as dpm of ^{3}H-GAG per 10^3 cells ± SD, with the cell numbers determined using a haemocytometer following trypsinization.

Assay of fibroblast fibronectin production

Confluent microcultures were used to assess the fibronectin production with the cultures exposed to ascorbic acid for the last 24 h of culture. The fibronectin content of the pooled supernatant media from the wells was determined using a commercially available immuno-turbidimetric assay kit (Boehringer-Mannheim, Indianapolis, IN, U.S.A.) as previously described,[13-15] and was reported as μg per 10^5 cells.

Assay of fibroblast collagenase production

Confluent microcultures treated as described previously for the assay of fibronectin production were also used to assess collagenase activity. However, the terminal 48 h incubations were performed in DMEM alone as the FCS interfered with the subsequent assay of the collagenase in the supernatant. Collagenase activity was measured by the solubilization of ^{14}C-labelled rat (type 1) collagen fibrillar gels using methods previously reported [13-15,18] and modified to assay larger volumes over a longer period of time. The supernatants were assayed in triplicate and the average results reported as mU of collagenase per 10^5 cells ± SD with 1·0 U defined as the amount of collagenase digesting 1·0 μg of collagen per min.

RESULTS

In vitro proliferation of keloid and scleroderma fibroblasts

The addition of 20% FCS to cultures of human keloid and scleroderma fibroblasts resulted in increases of 926% and 1132%, respectively, in the number of cells compared to serum-free cultures which displayed only a nominal proliferation during the 4-day culture period (Table 1). The exposure of serum-containing fibroblast cultures to pentoxifylline (100–1000 μg/ml) resulted in a dose-dependent reduction in cell numbers, the highest concentration virtually negating the proliferative effect of serum on both keloid and scleroderma cells.

The anti-proliferative effect of pentoxifylline was not due to lethal toxicity on the seeded fibroblasts, as the number of trypan blue-positive dead cells in the pentoxifylline treated cultures never exceeded those in the untreated controls (data not shown). Similarly, pentoxifylline had no effect on the viability of confluent fibroblast cultures used to assess the

TABLE 1. Effect of pentoxifylline on serum-driven proliferation of keloid and scleroderma fibroblasts

Treatment	Pentoxifylline concentration (μg/ml)	Fibroblasts (Cells ± SD/0·45 cm^2)	
		Keloid	Scleroderma
DMEM	0	1400 ± 15	1420 ± 15
DMEM + 20% FCS	0	12,960 ± 290	16,080 ± 460
	100	9450 ± 710	10,540 ± 410
	500	5960 ± 560	4980 ± 390
	1000	3540 ± 290	2960 ± 290

TABLE 2. Effect of pentoxifylline on constitutive and serum-driven collagen production by keloid and scleroderma fibroblasts

Pentoxifylline concentration (μg/ml)	^{3}H-collagen dpm ± SD/10^3 fibroblasts			
	Keloid		Scleroderma	
	DMEM	DMEM + 10% FCS	DMEM	DMEM + 10% FCS
0	47·3 ± 2·7	121·8 ± 3·4	39·0 ± 3·0	150·5 ± 9·6
100	35·9 ± 3·2	136·6 ± 2·3	39·8 ± 5·1	141·4 ± 3·1
500	8·0 ± 2·7	76·7 ± 4·2	32·1 ± 3·2	100·8 ± 15·0
1000	2·4 ± 1·4	59·6 ± 1·3	21·3 ± 2·1	60·1 ± 3·8

synthetic activities of these cells. However, pentoxifylline did cause a minor reduction in the cell number (10–20%) of these slowly proliferating confluent cultures during the 2-day assay period in the presence of 10% FCS. The results therefore have been quantitated on a per cell basis.

In vitro production of matrix components by keloid, scleroderma and morphoea fibroblasts

The addition of pentoxifylline (100–1000 μg/ml) to serum-free cultures of keloid and scleroderma fibroblasts resulted in a dose-dependent reduction in collagen production, with the keloid cells being more sensitive than the scleroderma cells at all concentrations (Table 2). The addition of pentoxifylline to serum-containing keloid or scleroderma fibroblast cultures similarly resulted in a dose-dependent reduction in collagen production with the highest concentration virtually negating the enhancing effect of 10% FCS on the production of extracellular collagen. The addition of 1000 μg/ml of pentoxifylline (PTX) to cultures of fibroblasts derived from two additional scleroderma patients and to fibroblasts derived from the inflammatory and fibrotic areas of a patient with morphoea resulted in the inhibition (45–93%) of constitutive and serum-driven collagen synthesis (Table 3).

TABLE 3. Effect of pentoxifylline on constitutive and serum-driven collagen production by morphoea and scleroderma fibroblasts

Fibroblast source	^{3}H-collagen dpm ± SD/10^3 fibroblasts			
	DMEM	DMEM + PTX	DMEM/FCS	DMEM/FCS + PTX
Morphoea				
Inflammatory	65·1 ± 8·0	4·3 ± 1·0	165·4 ± 2·4	49·5 ± 3·3
Fibrotic	54·3 ± 3·7	30·3 ± 6·2	144·5 ± 8·6	35·9 ± 5·7
Scleroderma				
Line 2	31·1 ± 2·3	9·4 ± 0·7	85·1 ± 0·8	47·3 ± 2·6
Line 3	21·4 ± 0·2	8·9 ± 0·4	50·1 ± 3·5	25·2 ± 0·4

TABLE 4. Effect of pentoxifylline on constitutive and serum-driven glycosaminoglycan production by keloid and scleroderma fibroblasts

Pentoxifylline concentration (μg/ml)	^{3}H-glycosaminoglycan dpm ± SD/10^3 cells			
	Keloid		Scleroderma	
	DMEM	DMEM + 10% FCS	DMEM	DMEM + 10% FCS
0	35·7 ± 2·0	57·7 ± 1·7	25·8 ± 0·5	34·8 ± 2·4
100	25·9 ± 1·3	50·6 ± 4·7	22·7 ± 3·0	35·3 ± 3·4
500	19·3 ± 1·4	31·2 ± 1·3	20·6 ± 2·5	13·2 ± 0·9
1000	4·0 ± 0·7	25·8 ± 2·4	19·4 ± 0·6	9·5 ± 1·2

Fibroblasts from keloid and scleroderma exposed to pentoxifylline (100–1000 μg/ml) under serum-free conditions were inhibited in a dose-dependent manner as regards their ability to produce GAG (Table 4). The addition of pentoxifylline to serum-containing fibroblast cultures also resulted in a dose-dependent reduction in GAG production. The addition of pentoxifylline to cultures of fibroblasts from two additional scleroderma patients and to fibroblasts from the inflammatory and fibrotic areas of morphoea resulted in a similar inhibition (45–71%) of constitutive and serum-driven GAG synthesis (Table 5).

The addition of pentoxifylline (500 μg/ml) to serum-free keloid or scleroderma fibroblast cultures resulted in a significant reduction in the extracellular fibronectin production. This was reduced from 2·03 μg/10^5 cells to 0·57 μg/10^5 cells in the keloid fibroblasts, and from 0·34 μg/10^5 cells to 0·12 μg/10^5 cells in the scleroderma fibroblasts. Similarly, 500 μg/ml pentoxifylline reduced fibronectin production in the presence of 10% FCS from 2·44 μg/10^5 cells to 1·83 μg/10^5 cells for keloid fibroblasts and from 1·84 μg/10^5 cells to 1·26 μg/10^5 cells for scleroderma fibroblasts.

TABLE 5. Effect of pentoxifylline on constitutive and serum-driven GAG production by morphoea and scleroderma fibroblasts

Fibroblast source	^{3}H-glycosaminoglycan dpm ± SD/10^3 fibroblasts			
	DMEM	DMEM + PTX	DMEM/FCS	DMEM/FCS + PTX
Morphoea				
Inflammatory	35·0 ± 2·8	10·5 ± 1·4	44·7 ± 3·5	14·8 ± 2·3
Fibrotic	55·5 ± 6·1	17·3 ± 1·2	62·7 ± 8·7	24·7 ± 1·3
Scleroderma				
Line 2	30·8 ± 0·3	16·9 ± 0·9	42·6 ± 2·3	20·2 ± 0·2
Line 3	50·2 ± 0·2	15·4 ± 0·4	43·8 ± 6·2	12·8 ± 1·3

TABLE 6. Lack of effect of pentoxifylline on collagenase activity in keloid and scleroderma fibroblasts

Pentoxifylline concentration (μg/ml)	Collagenase mU ± SD/10^5 cells	
	Keloid	Scleroderma
0	11·81 ± 0·65	18·48 ± 0·68
100	13·13 ± 1·28	19·22 ± 2·60
500	10·86 ± 2·50	16·26 ± 1·95
1000	12·50 ± 0·38	18·79 ± 3·10

In vitro collagenase activity produced by keloid and scleroderma fibroblasts

Exposure to pentoxifylline (100–1000 μg/ml) failed to alter the collagenase activity produced by fibroblasts from keloid and line 1 scleroderma as shown in Table 6.

DISCUSSION

Recent studies suggest that cytokines may be responsible in fibrotic skin diseases for activating excessive biosynthesis of the extracellular matrix which is characteristic for these disorders.[19,20] The use of cytokines in the treatment of these conditions has not as yet been fully developed,[7] and alternative treatments are required. The purpose of this study was to determine whether pentoxifylline inhibited the activities of fibroblasts derived from fibrotic skin, as we had previously found with those derived from normal adult skin.[15]

In our present study, we have demonstrated that *in vitro* exposure to pentoxifylline caused a dose-dependent inhibition of collagen, fibronectin and GAG production by fibroblasts derived from keloids and the skin of patients with scleroderma and morphoea. This was observed both in the absence and presence of 10% FCS. *In vitro* exposure to pentoxifylline also resulted in a marked reduction in the proliferation of fibroblasts derived from both keloids and from patients with scleroderma. However, it had no effect on collagenase activity by these fibroblasts. These results differ slightly from what we have previously reported for normal fibroblasts,[15] in that pentoxifylline only inhibited the collagen, fibronectin and GAG production of one normal fibroblast line studied in the presence of FCS and pentoxifylline enhanced its elaboration of collagenase activity. However, subsequent studies on three additional normal fibroblast lines indicate that most fibroblasts derived from normal skin have a degree of responsiveness to pentoxifylline comparable with those derived from fibrotic skin, with pentoxifylline inhibiting collagen, fibronectin and GAG production both in the absence and presence of FCS, but it has no effect on the collagenase activity.

The inhibitory effects of pentoxifylline on the proliferation and biosynthetic functions of fibrotic-derived and normal-derived fibroblasts does not appear to be due to a generalized toxic effect of pentoxifylline on fibroblasts, as both control and pentoxifylline-treated cultures contained similar low numbers of non-viable cells. Pentoxifylline is selective in its inhibitory control of protein synthesis by fibroblasts, in that no effect was observed on the collagenase activity. Also pentoxifylline in a concentration of 1000 μg/ml does not effect either the constitutive or cytokine-induced production of interleukin 6 m-RNA or protein, or cytokine-induced expression of the major histocompatibility antigen HLA-DR or intercellular adhesion molecule-1 or constitutive tumour necrosis factor-α-receptor expression by fibroblasts from normal or fibrotic skin.

This study suggests that pentoxifylline may be a useful anti-fibrotic agent for the treatment of dermal fibrotic disorders. Intravenous administration of pentoxifylline may be useful in producing concentrations *in vivo* similar to what is found to be inhibitory to fibroblasts *in vitro*. High concentrations may also be achieved following intralesional injection or selective tissue perfusion. Oral administration of pentoxifylline produces serum concentrations of only 2–5 μg/ml of unmetabolized drug.[21] However, the effectiveness of pentoxifylline at this concentration on leucocyte function is enhanced *in vivo* as compared to *in vitro* (W.Novick, personal communication), and this may be the case also for skin fibroblasts. Thus, oral administration of pentoxifylline could result in an effective anti-fibrotic therapy.

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Analysis of side-effects of medium- and low-dose cyclosporin maintenance therapy in psoriasis

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SUMMARY

The side-effects of long-term cyclosporin A (CyA) treatment in 26 patients with severe psoriasis were evaluated. These patients had a mean PASI score of 30·2 and were treated with CyA for between 7 and 37 months (mean 19·5 months). There were three groups according to the dose of CyA, < 2 mg/kg per day, 2–3 mg/kg per day and > 3 mg/kg per day. In all three groups, CyA was found to be equally effective. Treatment with CyA was discontinued in 12 of the 26 patients because of nephrotoxicity and/or development of hypertension. One was in the < 2 mg/kg per day group, three were in the 2–3 mg/kg per day group and eight in the > 3 mg/kg per day group. There was no hepatotoxicity with CyA treatment. One patient developed two squamous cell carcinomas of the skin.

Cyclosporin A (CyA) was first isolated in 1973[1] and was found to be effective in prolonging the survival of grafted tissue.[2,3] There is evidence that CyA induces immunosuppression by effecting the responses of resting T lymphocytes, as reviewed by Bloemena.[4] Recently, it was demonstrated that the CyA binding protein, cyclophilin, is identical to peptidyl-prolyl *cis-trans* isomerase.[5,6] These findings may contribute to the understanding of the early intracellular events in T-cell activation that are suppressed by CyA.

Psoriasis, which may be a T-cell mediated disease,[7] was first successfully treated with CyA in 1979.[8] Several studies have indicated that this drug is most effective for plaque psoriasis and also for palmoplantar pustular psoriasis.[9–22] However, so far the experience with CyA in the long-term therapy of psoriasis is limited.[23,24] Apart from the side-effects of hypertension and impaired renal function[20] little is known about the long-term side-effects in the treatment of psoriasis. We analysed the data of 26 patients with psoriasis who were treated with CyA for a mean period of 19·5 months.

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PATIENTS AND METHODS

Patients

Twenty-six patients with severe psoriasis who had treatment with CyA for a mean period of 19·5 months (range 7–37 months) were studied. One patient also had palmoplantar pustulosis in addition to plaque psoriasis. All the patients had previously received topical treatment and apart from five, they had all had systemic treatment with oral psoralens and UVA (PUVA) and/or methotrexate and/or etretinate. None of the patients had systemic treatment in the month before starting therapy with CyA.

Cyclosporin treatment

Treatment with CyA was given for at least 7 months without interruption. All the patients were initially treated with 3 mg/kg per day of cyclosporin and according to the clinical response the dosage was then adjusted. If the treatment was discontinued it was stopped for no longer than 4 weeks. The dose was given either once or twice daily. The clinical evaluation before and during therapy with CyA was made using the Psoriasis Area and Severity Index (PASI).[25] At monthly intervals patients were evaluated as to their PASI score and for side-effects. Laboratory investigations were also carried out. Hypertension was defined as a diastolic blood pressure of 95 mmHg or more and/or a systolic pressure of > 150 mmHg. Laboratory investigations included a full blood count, renal and liver function tests. The trough concentration of CyA in the blood was determined by a radioimmunoassay.

RESULTS

According to the mean dose of CyA given during the treatment period, there were three groups formed as shown in Table 1. The three groups were comparable for the mean age, initial PASI score and the maximal reduction of the PASI and the duration of treatment. All the clinical data from the CyA-treated patients were analysed.

Blood pressure

There was a dose-related effect on the diastolic blood pressure, as shown in Figure 1. During the first 18 months of treatment with CyA there was an increase in the mean diastolic blood pressure which appeared to be dose-related (Table 2). The mean increase of diastolic pressure in the 10 patients treated with CyA > 3 mg/kg per day was 16 mmHg and hypertension occurred in five of

TABLE 1. Comparison of three different groups of CyA-treated psoriasis patients; number of patients (n) in each group, mean and standard deviation (SD) of age, PASI, maximal achieved PASI reduction and duration of CyA treatment

Mean CyA dosage (mg/kg per day)	n	Age (years)		PASI		PASI-reduction (%)		Treatment-period (months)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
0–2	5	55·4	15·8	21·2	5·8	87·6	10·9	19·6	8·5
2–3	10	51·2	15·6	27·8	8·4	95·0	3·6	16·5	6·9
> 3*	11	48·8	13·8	36·5	14·1	87·5	17·8	21·3	9·0

* Range 3–12·4 mg CyA/kg per day.

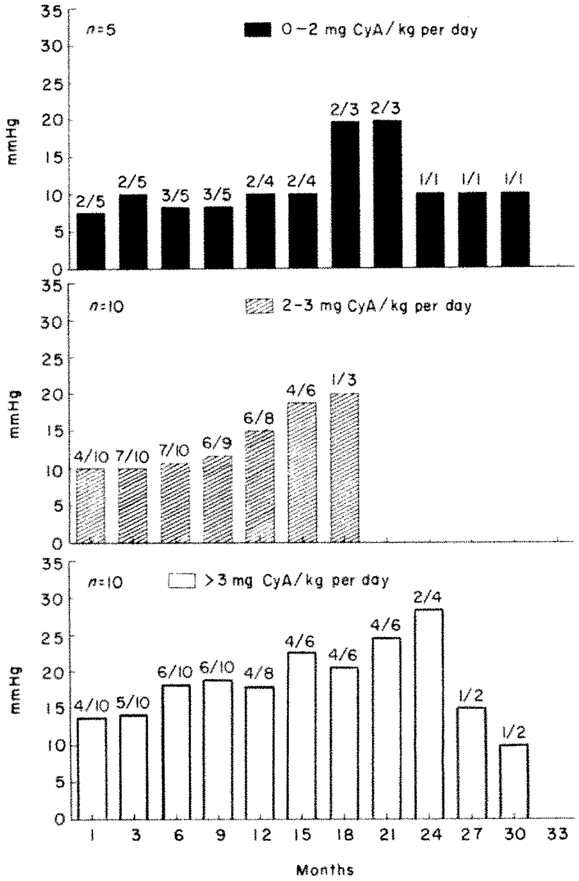

FIGURE 1. The mean increase of diastolic blood pressure (mmHg) versus the treatment time (months) is given for each CyA dose group. The numbers above the columns indicate the number of patients with an increase of diastolic blood pressure versus the number of patients treated with CyA.

TABLE 2. Post-treatment evaluation of all patients

	CyA dosage (mg/kg per day)		
Mean increase of:	0–2	2–3	>3
Diastolic BP (mmHg)	12	11	16
Systolic BP (mmHg)	22	8	15
Creatinine (μmol/l)	9	18	26
Potassium (mmol/l)	0·7	0·5	0·7
Alkaline phosphatase (mmol/l)	14	10	6

Data are presented for all patients after completion of CyA treatment. BP, blood pressure.

them. There was no definite correlation between the dose of CyA and the mean increase of the systolic blood pressure (Table 2). However, those patients treated with >3 mg/kg per day developed a time-related increase in the systolic blood pressure (data not shown).

Creatinine

There was a dose- and time-related effect on the mean serum creatinine level in the patients. This was defined as an increase of 110% above the baseline value for the serum creatinine. After 2 years of treatment, all the patients still on therapy had an increase in their serum creatinine (Fig. 2). This was most marked in those patients treated with >2 mg/kg per day of CyA.

Potassium

An increase in the level of potassium in the blood was found in almost all the patients treated with CyA (Table 2). The dosage of CyA >3 mg/kg per day produced in some patients hyperkalemia at a level >4·5 mmol/l (Table 3).

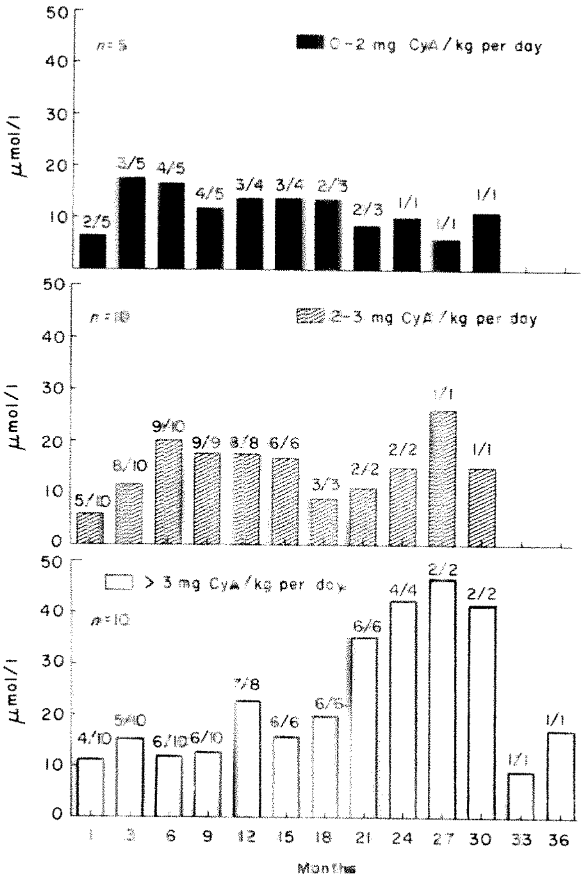

FIGURE 2. The mean increase of serum creatinine (μmol/l) versus the treatment time (months) is given for each CyA dose group. The numbers above the columns indicate the number of patients with an increase of serum creatinine versus the number of patients treated with CyA.

TABLE 3. Summary of objective and subjective adverse effects, arranged according to CyA dosage. The numbers correspond to the number of patients with an adverse effect (n = number of patients in corresponding CyA-dosage group).

Adverse effect	CyA dosage (mg/kg per day) 0–2 ($n=5$)	2–3 ($n=10$)	>3 ($n=11$)
Objective signs			
Hypertension	2	1	5
Nephrotoxicity	2	7	9
Hyperkalemia	3	4	5
Squamous cell carcinoma	0	0	1
Febris e.c.i.	0	0	1
Tremor	0	3	0
Gingiva hyperplasia	0	1	0
Hypertrichosis	0	2	2
Subjective signs			
Myalgia	1	2	2
Gastrointestinal upset	1	0	2
Nausea	2	2	2
Vertigo	0	0	2
Fatigue	1	2	3
Hyperhidrosis	1	0	0
Headache	0	2	4

Alkaline phosphatase

During treatment with CyA there was an increase in the level of alkaline phosphatase in the majority of the patients. However, this did not correlate with the dose of CyA (Table 2).

Adverse effects

Treatment with CyA was discontinued in 12 of the 26 patients because of nephrotoxicity and/or hypertension (Table 3). One patient was from the < 2 mg/kg per day group, three from the 2–3 mg/kg per day group and eight from the > 3 mg/kg per day group. There were no other consistent abnormal laboratory findings in the patients treated with CyA, and in particular there was no hepatotoxicity from the drug. On stopping the treatment with CyA, the hypertension and signs of nephrotoxicity resolved.

An overview of all the side-effects as noted is shown in Table 3. In one patient two squamous cell carcinomas of the skin were diagnosed; this patient had been treated in the past with PUVA, methotrexate and etretinate. Several patients, especially females with dark hair, complained about hypertrichosis. There were other well-known CyA-related side-effects in the patients such as gastrointestinal disturbances and tremor. Several patients suffered from myalgia and fatigue. These adverse effects subsided within days of discontinuing CyA treatment.

DISCUSSION

In our study it was noted that patients with severe psoriasis appeared to require a higher dose of CyA than patients with less involvement. However, the analysis of the PASI of these patients and of the dose of CyA indicated that this index did not predict the dose of CyA that is required. This supports our hypothesis[23] that each individual patient has a critical dose of CyA that is required to clear their psoriasis. It can be envisaged that above a certain dose of CyA, the reduction in PASI score is independent from the dose given. Our results on long-term treatment indicated that tachyphylaxis with CyA does not occur in psoriasis.[23,24]

A major problem in the analysis of our data is the difference in the treatment periods. Only those patients with either acceptable or no significant adverse effects remained on treatment with CyA. Because of this selection there is a misleading decline in blood pressure and the serum creatinine after 2 years. Hypertension developed in eight patients, mainly in those who were on CyA in a dosage > 3 mg/kg per day. A reduction in the dose of CyA because of hypertension or because of an elevated serum creatinine or hyperkalemia resulted in the exacerbation of psoriasis in 12 of the patients. The use of anti-hypertensive drugs was unsatisfactory in our patients.

In contrast to the reports on renal transplant patients,[26] we found that in our patients with psoriasis there was a progressive increase in the diastolic blood pressure and the levels of creatinine and potassium during continuous long-term therapy with CyA.

Although high-dose treatment with CyA may lead to hepatotoxicity in renal transplant patients,[26] we found no significant changes in the liver function tests in our patients. The rise in alkaline phosphatase seen in our study was probably due to an isoenzyme of bone origin and not related to liver damage.[27,28] In our study none of the patients had any lymphoproliferative disorder, though malignant lymphomas have been reported in patients on therapy with CyA. As recently reported, one of our patients with psoriasis developed two squamous cell carcinomas during treatment with CyA,[29] but it is difficult, in view of the previous treatment, to establish the precise role of the drug in the development of the skin cancers. However, the occurrence of skin malignancies emphasises the importance of a complete examination before and during treatment with CyA. Recently, squamous cell carcinomas have been reported in patients on CyA therapy for non-malignant skin disorders,[30] and an increased frequency of epidermal dysplasia was found in renal transplant patients treated with CyA.[31]

This study confirms that CyA is most effective in clearing psoriasis and in maintaining remission. Therapy with CyA is, however, restricted by time- and dose-related nephrotoxicity, hypertension and hyperkalemia.

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White piedra: evidence for a synergistic infection

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SUMMARY

To determine the relative roles of coryneform bacteria and *Trichosporon beigelii* in the pathogenesis of genital white piedra, scrotal hair from 10 subjects was studied. Hairs were examined by light microscopy to determine the relative proportions of each organism, and were also cultured for coryneforms and yeasts. Histologically, hair nodules from five out of nine cases showed a mixture of yeasts and bacteria, four had bacteria alone, and none showed yeast alone. Five strains of *T. beigelii* were cultured, two strains of *Saccharomyces cerevisiae* and 22 strains of coryneforms. The isolates were tested for synergism by a plate-overlay method. Growth of coryneforms occurred over and around sections of the plate inoculated with *T. beigelii* but not around the control yeast, *S. cerevisiae*. There were strain differences in the stimulatory response of both *T. beigelii* and coryneform strains. In reverse experiments coryneforms did not enhance growth of *T. beigelii*. It was concluded that white piedra is a mixed infection caused by the synergistic action between *T. beigelii* and a specific coryneform bacteria resulting in invasion of the hair cuticle and cortex.

White piedra was originally described in 1865 by Beigel[1] who discovered white nodules adherent to the hair of a wig. In 1902, Vuillemian found similar nodules on the hair of a moustache and isolated a yeast-like fungus which he named *Trichosporon beigelii*. Over the years white piedra has been thought primarily to affect the hair of the scalp, beard, and moustache, but has also been found to affect the eyelash, eyebrow, axillary and especially perigenital hair. Clinically, it appears as white to tan, green or reddish nodules adherent to the shaft of terminal hairs. They are often barely visible and may be either discrete or coalesced into sleeve-like concretions.[2–5] White piedra was considered to be very rare in the United States until 1986 when a 40% prevalence was found in patients living in Houston, Texas.[6] In the same study, coryneform bacteria were commonly found among the hair concretions.

Trichomycosis pubis is considered to be a variant of trichomycosis axillaris, a superficial

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infection of axillary hair by coryneform bacteria resulting in the formation of small nodules adherent to the hair shaft.[7] While axillary involvement has been reported to occur in as many as one-third of normal adults,[8] the prevalence of pubic hair infection is thought to be much lower. An incidence of between 26 and 35% was reported for the former and only 2·6% for the latter.[9] The aim in this project was to study, in detail, the relative role of coryneform bacteria and yeasts in the pathogenesis of genital white piedra and to compare it with trichomycosis pubis.

METHODS

Morphological studies

Using alcohol-cleansed scissors, scrotal hairs were clipped from nine subjects with genital hair nodules and one control subject free of hair nodules. All subjects gave informed consent. The specimens were examined at × 10 magnification using a stereoscopic microscope to verify the presence of nodules on the hair shaft. A 10% potassium hydroxide preparation was examined at × 100 and × 1000 magnifications and the presence of bacteria and/or yeast forms noted. The hair was embedded in plastic; thin sections were stained with toluidine blue and were examined at low and high powers, and under oil immersion.

Microbiology

Additional hairs from the same subjects, were soaked overnight in phosphate-buffered saline pH 7·2 containing 0·1% Tween 80 to soften the encrustations. These were removed by scraping and inoculated onto Casman's sheep blood agar plates, incubated at 35°C for cultivation of bacteria and onto Sabouraud glucose agar (SGA) slants and incubated at 25°C for cultivation of fungi. All bacteria were identified by routine diagnostic methods.[10] Yeasts were screened morphologically and for urease production and further identified by the API Yeast Ident system.

Synergism studies

A plate-overlay method was used to test for synergism between *T. beigelii* strains and coryneform bacteria. *T. beigelii* isolates were spot inoculated onto SGA plates. Coryneform bacteria, cultured on Casman's sheep blood agar for 3 days at 35°C, were suspended in 0·85% NaCl and the suspensions were standardized to the optical equivalent of the MacFarland tube no. 1. An inoculum of 0·1 ml of suspension was added to 10 ml of molten 1·5% agar (Bacto agar, Difco Laboratories, Detroit, MI, U.S.A.) in tap water at 48°C and overlayed on the SGA plates which were incubated at 35°C for 5 days. In tap water agar, minute colonies are formed but the medium is incapable of supporting further growth of the coryneforms. The appearance of a zone of confluent growth surrounding the point of inoculation of *T. beigelii* was therefore defined as synergistic growth. Other media for overlays were tried including distilled water, buffered saline at pH 7·2, and 0·85% saline, but zones of stimulation of growth were best demonstrated using tap water agar. Synergism experiments were also done with four *T. beigelii* strains isolated from our previous study, one strain of *Candida albicans* and one of *S. cerevisiae* (Baker's yeast). Six additional coryneform bacteria from the previous study and seven culture collection strains of *Corynebacterium* spp. and related organisms were also examined (Table 1).

TABLE 1. Bacteria and their source

Bacteria	Source
C. diptheriae	Dr J. Clarridge*
C. pseudotuberculosis	Dr J. Clarridge
Corynebacterium JK	Dr J. Clarridge
Rhodococcus equi	Dr J. Clarridge
C. renale	ATCC 19412
C. pilosum	ATCC 29592

* Veterans Administration Hospital, Houston, Texas, U.S.A.

RESULTS

Histology

Macroscopically, all the specimens looked similar with lightly coloured nodules adherent to the shaft of the terminal hair. Toluidine blue imparted a red colour to the fungal walls but stained the bacteria blue. Under low power light microscopy (Fig. 1), it was easy to identify the fungi but under higher magnification oil immersion (Fig. 2) was necessary to appreciate the bacteria adherent to the hair shaft. In some cases, both bacteria and fungi had invaded the cortex (Fig. 3). Potassium hydroxide examination of the affected hair was not useful in identifying bacterial infection although the characteristic masses of the yeast forms of *T. beigelii* could easily be seen.

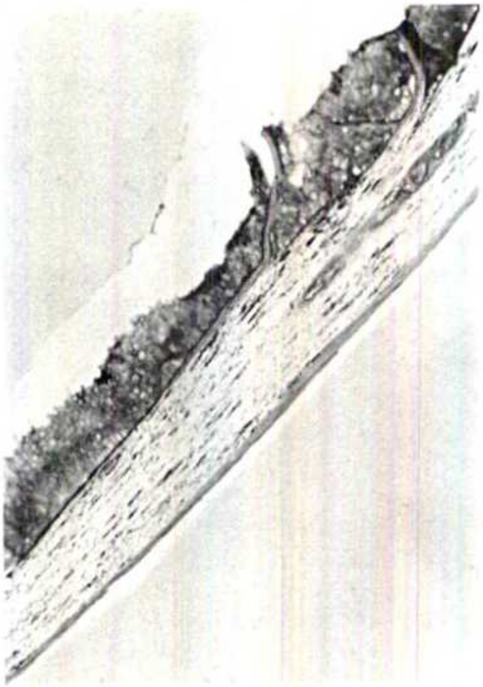

FIGURE 1. Thin section of affected hair imbedded in plastic. Yeast forms of *Trichosporon beigelii* are readily apparent but bacteria are not discernible (toluidine blue, × 400).

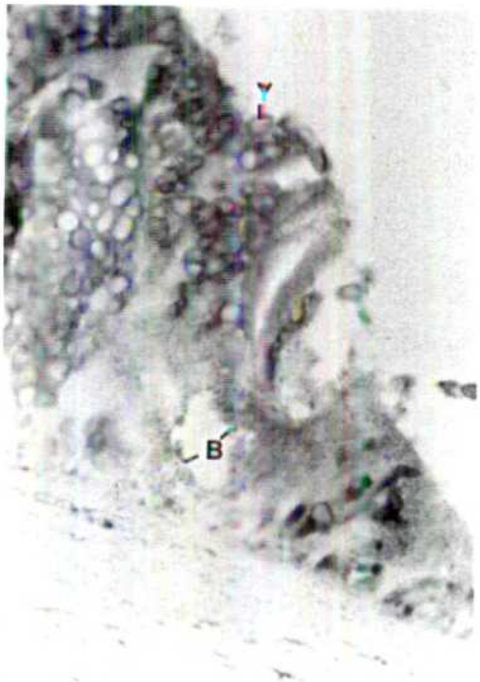

FIGURE 2. Thin section of hair showing elevation of the cuticle by invading organisms. Y, yeast forms and B, bacteria (toluidine blue, × 1000).

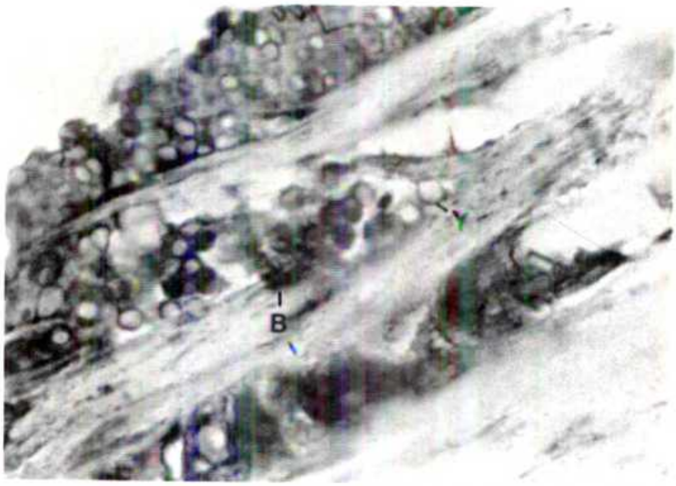

FIGURE 3. Thin section of hair showing invasion of the hair cortex by both bacteria (B) and yeast (Y) (toluidine blue, × 1000).

Both bacteria and *T. beigelii* were observed in five out of 10 subjects; four subjects demonstrated coryneform bacteria alone; while in the remaining control case, no lesions were seen.

Microbiology

Table 2 summarizes the organisms isolated from each culture. *T. beigelii* was isolated from every

TABLE 2. Correlation between histology and cultures of infected hair

		Micro-organism cultured						
						Coryneforms		
Patient designation	Histology of hair	*T. beigelii*	Other yeast	*Staphylococcus epidermidis*	Gram negative rods	Lipophilic	Smooth	Group III
E-1	Y* + B†	+	−	+	+	−	−	−
E-2	Y + B	+	+	+	−	−	−	+ (2)
E-3	Y + B	+	−	−	+	−	−	+ (2)
E-4	No lesion	−	−	+	−	+ (1)	−	−
E-5	B	−	−	+	−	−	−	−
E-6	B	−	+	−	−	−	+	−
E-7	Y + B	+	+	−	−	−	−	+ (3)
E-8	Y + B	+	−	+	−	−	+	+ (1)
E-9	B	−	−	+	−	+ (1)	+ (3)	−
E-10	B	−	−	−	−	+ (1)	+ (3)	−

* Y, Yeast. † B, Bacteria. ( ), No. of strains. +, Organism present. −, Organism absent.

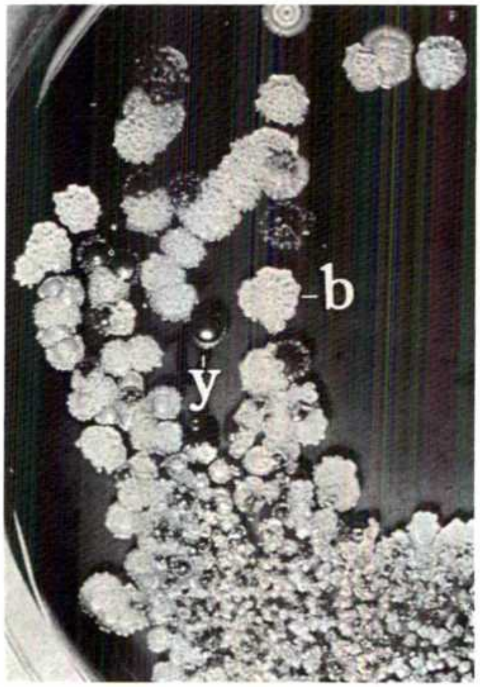

FIGURE 4. Culture from direct plating of a crushed hair nodule showing distinctive colonial morphology of Group III coryneform bacteria grown on Casman's sheep blood agar at 37°C for 5 days (× 5·4). B, bacterial colony; Y, *T. beigelii* colony which is a smooth colony when grown on blood agar at 37°C.

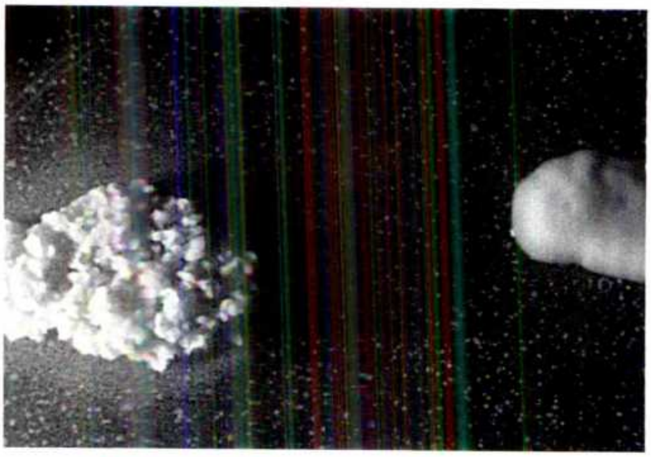

FIGURE 5. Synergistic growth pattern of coryneforms around colonies of *T. beigelii* (left). No stimulation of growth occurs around the colony of *Saccharomyces cerevisiae* (right).

specimen where yeasts were visualized. In two patients, E-2 and E-7, *S. cerevisiae* was isolated as well. This organism was cultured from E-6, although no yeasts were seen histologically in the hair lesions. Coryneform bacteria were cultured from nine of the 10 specimens including E-4, in which there were no lesions. Although coryneforms were seen histologically in E-1, none were cultured. Because of the lack of a definitive taxonomic scheme for coryneform bacteria, they were divided by their colonial morphology into three groups: the lipophilic small colony type, the smooth creamy large colony type and the third designated as Group III. The organisms in Group III have a distinctive colonial morphology (Fig. 4) when isolated with *T. beigelii* on Casman's sheep blood agar grown at 37°C. Under these cultural conditions, *T. beigelii* forms a smooth dark colony unlike the distinctive colony formed on SGA.

Synergism

A synergistic bacterial growth pattern was observed when *T. beigelii* was cultured with certain isolates of coryneform using the overlay method. In Figure 5, enhanced growth of coryneform bacteria is seen surrounding the *T. beigelii* colony. All strains of *T. beigelii* enhanced growth of

TABLE 3. Synergistic growth of coryneforms in the presence of *T. beigelii*

Bacteria tested	No. strains showing synergism
Lipophilic coryneforms	0/3
Smooth coryneforms	3/16
Group III coryneforms	9/14
C. diphtheria	0/1
C. renale	0/1
C. pilosum	0/1
C. JK	0/1
Group G-II	0/1
Rhodococcus equi	1/1

susceptible bacteria, but to a variable degree. *S. cerevisiae* had no effect upon the growth of coryneforms while *Candida albicans* was inhibitory to bacterial growth. Table 3 summarizes the stimulation of growth of the various groups of coryneform bacteria when cultured with *T. beigelii*. Synergistic growth of lipophilic coryneforms and culture collection species of *Corynebacteria* was absent. The growth of nine out of 14 isolates of Group III coryneforms was enhanced as was that of the single strain of *Rhodococcus equi*.

DISCUSSION

Although chemical methods have increased the understanding of the taxonomy of Gram-positive non-sporeforming bacteria,[11] those isolated from normal skin are, as yet, not well defined. For descriptive purposes in this study, the designations of large colony, lipophilic and Group III were chosen. Lipophilic coryneforms, which grow poorly on most routine bacteriological media unless supplemented with lipid such as Tween 80, are well recognized by skin microbiologists[12] as are some of the common large colony types. The bacteria associated with white piedra lesions, designated Group III, can, on culture, be readily differentiated. These organisms form pinpoint colonies after 3 days' incubation at 35–37°C, but after further incubation up to 5 days, the characteristic convex colony with papillate surface and crenated edge develops with a distinct malodour. Therefore, with an incubation time of 48 h, these organisms would not be observed on routine cultures even though they are not fastidious. Characterization is currently in progress and the results of cell-wall analysis, fatty acid composition and biochemical tests indicate that this is a new species of *Brevibacterium*.[13]

The frequent isolation of Group III coryneforms with *T. beigelii* in our previous report[6] led to the expectation that a specific relationship between these coryneforms and *T. beigelii* would be found in this more detailed study. Indeed, of the five specimens with *T. beigelii*, Group III coryneforms were isolated from four of these. In the remaining case, E-1, coryneform bacteria were visualized histologically, but were not cultured. In this case, the heavy growth of *E. coli* and *Staphylococcus epidermidis* may have prevented the isolation of the slower growing coryneforms. Although there were only four cases of the combination of *T. beigelii* and the distinctive Group III coryneforms, these organisms were not isolated from the other cases which yielded only coryneforms from the hair lesions. This association, in combination with the *in vitro* growth data, suggests that this is a specific mixed infection. This report shows (supporting previous studies[9,14]), that a variety of coryneforms can colonize hair and invade the cuticle. The question is whether the extent of invasion into the cortex as shown in Figure 3, is facilitated by the synergistic activity of the two organisms.

Although we have been able to demonstrate stimulation of bacterial growth in the presence of *T. beigelii* and not the reverse, some mutually synergistic relationship appears to exist between the yeast and the Group III. Fungal by-products of metabolism may provide nutrients to augment bacterial growth. Proteolytic activity, which is one of the biochemical features of Group III coryneform, may then facilitate hair shaft invasion by both organisms.

Much has been written on trichomycosis axillaris,[9,15,16] but little on trichomycosis pubis as an independent entity. The first study in the English literature was by White and Smith[17] who attributed the infection to an unknown coryneform. In one of their two patients they also cultured *T. beigelii* (as '*T. cutaneum*') but they considered this to be a contaminant. Both patients had a clinical picture consistent with white piedra. None of the literature on trichomycosis pubis makes any mention of attempting to culture for *T. beigelii*.

Culture results may be misleading. *T. beigelii* is usually inhibited by cyclohexamide[18] and does not grow readily on Mycosel or dermatophyte test medium (DTM), the most common culture media used by dermatologists. It is best grown on SGA with chloramphenicol. Coryneforms are not routinely cultured, and identification of species is difficult. Because of the difficulties in culturing these organisms, an exact diagnosis of white piedra or trichomycosis pubis may be impossible to make using routine culture methods.

Most reports on clinical studies of white piedra mention difficulty in eradicating the infection.[6,18,19] Temporary resolution can be achieved, but usually the infection rapidly recurs. This could be due to environmental factors, host susceptibility, inadequate treatment of all the involved pathogens and poor personal hygiene. Earlier reports speculated about the role of personal hygiene in the pathogenesis of both white piedra[3,5] and corynebacteria infections.[20] Treatment should be aimed at the coryneforms which are sensitive to Triclosan, an active ingredient in antibacterial soaps, and also to topical antibiotics such as Clindamycin or Erythromycin. It is doubtful that systemic antibiotics would have any effect on hair concretions.

In conclusion, we postulate that there are two diseases: trichomycosis pubis, caused by a variety of coryneforms, a counterpart of trichomycosis axillaris in the groin and white piedra, which is caused by the synergistic effect of *T. beigelii* and a specific coryneform. It is uncertain whether *T. beigelii* stimulates the growth of the coryneform which is the primary invader, by proteolytic activity, or if the organisms together are necessary to penetrate the hair. It is important to know, however, that the isolation of *T. beigelii* from hair is actually from the lesion as *T. beigelii* has also been found on normal skin in the perigenital area.[21,22] The criteria in making the diagnosis of genital white piedra are: (i) the presence of clinical disease, which includes nodules on hair shafts and a possible association with rash and pruritus or irritation; (ii) the presence of fungal elements and bacteria in the affected hair shaft seen on microscopic examination; and (iii) the growth of *T. beigelii* and the specific coryneform from affected hair on the appropriate media. Further studies are in progress to characterize the coryneform and also to determine the prevalence of *T. beigelii* on normal skin in the groin.

ACKNOWLEDGMENTS

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Ultrasound velocity in human fingernail and effects of hydration: validation of *in vivo* nail thickness measurement techniques

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SUMMARY

Distal nail thickness was measured using an electronic micrometer and both distal and proximal nail ultrasound times were recorded in 20 volunteers (10 male, 10 female) aged 20–39. The fingernail ultrasound velocity was 2.26×10^3 m/s (subject range 2.03–2.69) (analysis of variance technique). The proximal ultrasound transit time was greater than distal ultrasound transit time. In three volunteers, five micrometer and one distal midline ultrasound measurement of five nails were repeated on 10 occasions over 2 weeks. For the micrometer readings the average coefficient of variation was 5.3% (SD ± 2.4%), and for the ultrasound reading the average coefficient of variation was 4.0% (SD ± 1.3%).

To assess the influence of hydration, in five volunteers the distal nail micrometer thickness and the distal nail ultrasound transit time were measured on five nails before and after 30 min of immersion in water initially at 37°C. The mean distal ultrasound transmission time increased from 0.20 ± 0.04 μs to 0.22 ± 0.04 μs ($P < 0.001$) after water immersion. The micrometer measurements and ultrasound velocity did not change significantly (mean ultrasound velocity $= 2.01 \times 10^3$ m/s before, 2.04×10^3 m/s after immersion).

Instrumentation capable of measuring nail thickness accurately *in vivo* is needed to record disease status, e.g. in the yellow nail syndrome or in thin brittle nails, and to record changes in nails following therapy. Nail thickness measurements are needed to interpret the rate of nail-plate formation from the nail linear growth rate, and to interpret other nail physical properties such as flexibility.

Following the description of the measurement of ultrasound transmission time in human fingernails *in vivo*[1] there is a need to validate this technique, by determining the repeatability of

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the measurements and by estimating the normal range of nail ultrasound velocity *in vivo*. In addition the range of variation expected in repeated measurement of distal nail thickness by an adapted electronic micrometer needs to be established, as it is this measurement which is used as a standard for interpretation of ultrasound measurements. The previous estimate of human fingernail velocity was 1600–2800 m/s,[1] confirmed by Jemec and Serup.[2] The main aims of this study were to establish more exactly ultrasound velocity *in vivo*, and to determine the range of repeatability of nail ultrasound and micrometer readings.

The degree of hydration of nails is the single most important factor in influencing the physical properties of hardness, flexibility and brittleness. As the degree of nail hydration may vary considerably, knowledge of its effect on measurement methodology is important. Dried cadaver nails *in vitro* increase in weight by 18% after 30 min of immersion in water and become more flexible.[3] Nails are easier to cut after a bath, but the influence of water immersion *in vivo* on nails has not previously been formally studied. It is important to establish whether previous recent water immersion may influence ultrasound or micrometer readings.

Once these techniques have been validated it might then become possible to use ultrasound to measure nail thickness across the nail plate *in vivo*. The measurement of physical properties and the function of nails *in vivo* is more relevant but more difficult than *in vitro*,[4] and ultrasound may provide a useful additional objective technique. Measurement of nail thickness is of value in the assessment of therapy, in the further quantification of congenital and acquired nail defects and the effects of systemic diseases such as iron deficiency or uraemia on nail plate.

METHODS

Twenty healthy volunteers agreed to have their nails measured by ultrasound and micrometer. This study had received approval from the University Hospital of Wales Medical Ethical Committee. All 10 fingernails were studied on each of the 20 subjects (10 male, 10 female; aged 20–39, 10 aged 20–29 years, 10 aged 30–39 years). All the subjects were right handed.

The distal nail thickness was measured five times on each nail using an adapted Moore and Wright Micro 2000 electronic micrometer.[1] The site of measurement was within the middle third of the protruding nail. The tip of the 'anvil' on the micrometer, which is approximately 1 mm across, was placed as close as possible to the junction of the inferior border of the nail and underlying skin, but not so far in as to cause discomfort by compression of the underlying distal nail bed.

The ultrasound transmission time was measured using the Cutech Dermal Depth Detector, an 18 MHz centre frequency, 16 MHz band-width pulse-echo ultrasound system. An especially made conical ultrasound probe was used; this was designed to allow easier orientation of the probe at right angles to the curved nail surface by having a wider flat base (4-mm diameter), but retaining a standard central transmission hole (3-mm diameter). Water was used as the sonar medium; domestic 'Cling-Film' (R) was used to hold the water in place and a water based gel was used to provide contact between the probe and the curved nail surface.

On each nail an ultrasound transmission time was recorded in the nail midline distally so that the centre of the probe was either overlying or as near as possible to the site of micrometer measurement. In addition a further reading was taken also in the midline but approximately 3 mm from the proximal nail fold.

A typical ultrasound echo pattern displayed on the oscilloscope is shown in Figure 1. It was decided to standardize the echo time recorded as being that between the maximal 'down' deflection in the initial nail surface echo complex and the maximal 'up' deflection in the nail–

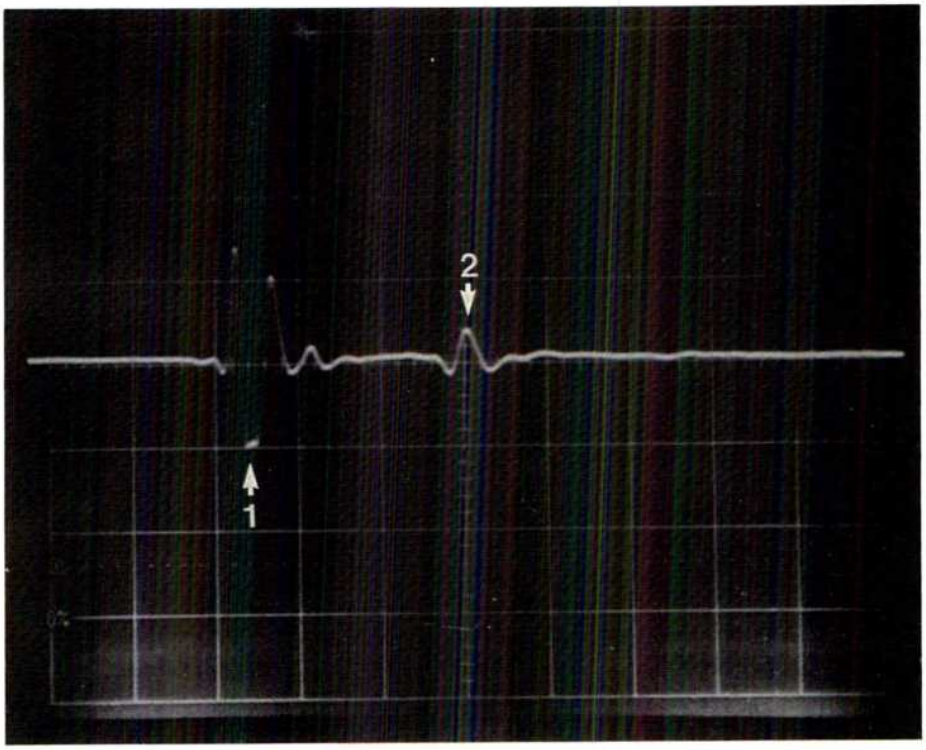

FIGURE 1. Oscilloscope display of ultrasound echo. The arrows point to the maximal 'down' echo (arrow 1) and the maximal reflected 'up' echo (arrow 2). The time between these points was recorded. The horizontal scale is 0·2 μs per large division. This figure is from an actual measurement of nail thickness of the distal part of a thumbnail *in vivo*.

epidermis interface echo complex, in order to take into account the expected phase reversal of the echo at the water–nail interface. In order to assess the variability of the above techniques, repeated readings were carried out daily on three male volunteers in the 30–39 year age range. On each occasion all five nails of the right hand were measured. Five distal micrometer readings and one distal ultrasound reading were taken from each nail as described above. In two volunteers 10 readings were taken and in the other volunteer eight readings taken within a 12-day period. The measurements were recorded on each occasion without reference to previous recorded results.

Assessment was made of the influence of hydration on nail thickness, ultrasound transmission time and ultrasound velocity. In five volunteers (males aged 30–39) one distal ultrasound reading and five micrometer readings were taken on each nail of the right hand immediately before and immediately after immersion of the hand and nails in water for 30 min. This time was chosen because measurements of *in vitro* water immersion suggest that almost maximal water uptake will have occurred by 30 min.[4] The temperature of the water at the start of the immersion ranged from 36–39°C, and at the end of the 30 min in each case the temperature had fallen by approximately 5°C.

The results were analysed using analysis of variance and Wilcoxon techniques.

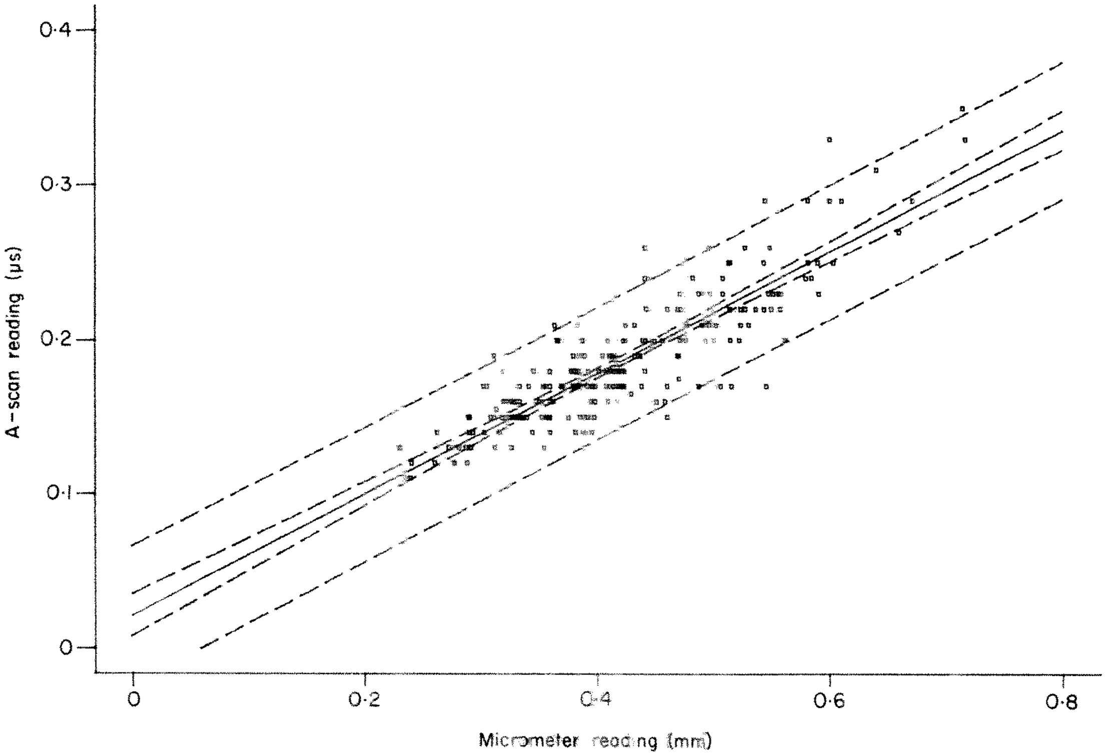

FIGURE 2. The relationship of distal ultrasound transmission time to distal micrometer measurements (200). The slope is 0·392 μs/mm. The outermost dotted lines represent two standard deviations from the mean and the inner dotted line represents one standard deviation from the mean.

RESULTS

Ultrasound velocity in nail

The relationship of the 200 measurements of ultrasound transit time to distal micrometer measurement is given in Figure 2. This shows the linear regression of the one-way ultrasound transit time on the micrometer thickness readings. This regression analysis was used to determine whether a linear relationship existed between the variables (ultrasound and micrometer), and to establish an estimate of the percentage variation of the derived individual velocity values. It should be noted that in undertaking regression analysis of two variables *a* and *b*, because of the fundamental concepts involved in calculating the slope of the graph, the results obtained are different depending on whether '*a*' or '*b*' is the independent variable. Hence the inverse of the slope of the regression of A-scan on micrometer readings is not the same as the slope of the regression of micrometer on A-scan readings. Clearly the independent variable in this case is the micrometer reading: however regression analysis was carried out in both ways. As the two graphs intercepted the '*y*' axis on both analyses, this indicated that there was no reason to believe that there was a systematic error in the ultrasound readings, for example that the wrong point on the oscilloscope trace was chosen.

Having established this, 200 paired readings were log transformed to improve the symmetry of the distribution. Each individual log velocity value was calculated, the mean taken and the

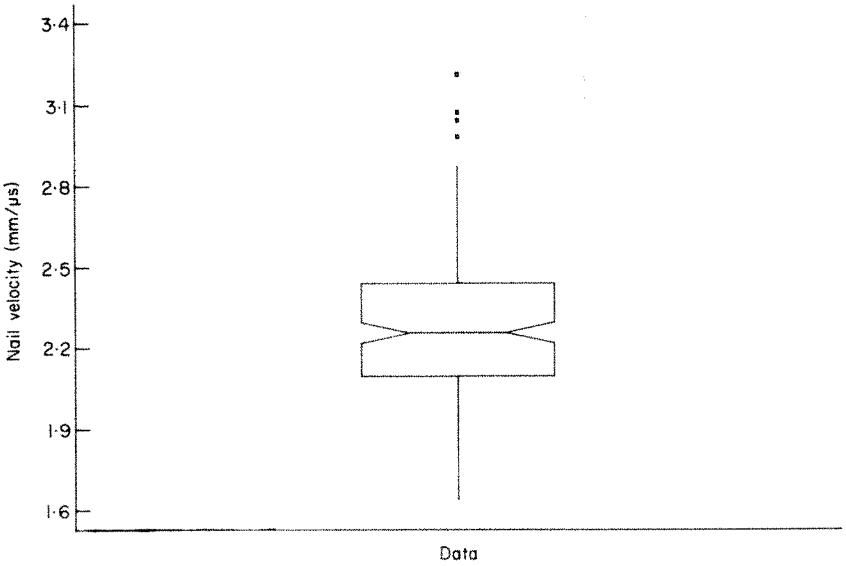

FIGURE 3. Notched box and whisker plot to show distribution of derived ultrasound velocities in 200 nails. In a notched box and whisker plot, the width of the box is proportional to the square root of the number of observations. The box contains the central 50% of the data points. The centre line represents the median value. The whiskers extend to the extreme values. Individual points beyond 1·5 times the box length are placed as individual adjacent values. The boxes are notched so that any pair of boxes with non-overlapping notches have significantly different median values at a level of $P = 0{\cdot}05$.

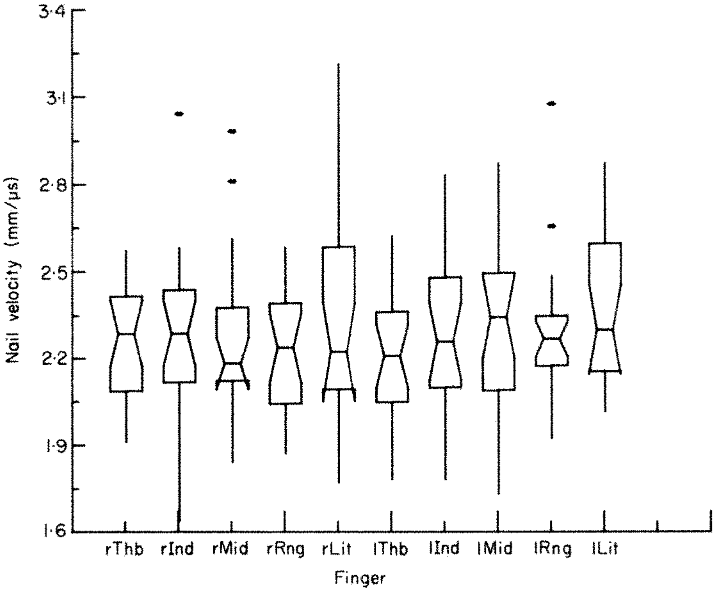

FIGURE 4. Notched box and whisker plot to show nail ultrasound velocity for each finger position from 20 subjects.

velocity mean derived from the anti-log of this. The mean ultrasound velocity (individual nail) derived in this way was $2{\cdot}26 \times 10^3$ m/s (range $1{\cdot}64$–$3{\cdot}21$) (Fig. 3).

A two-way analysis of variance testing for the effect of finger position and subject showed no significant effect of finger position on the velocity. The median nail ultrasound velocity for each finger position is given in Figure 4; range (individual nail position) of velocity $= 2{\cdot}18$–$2{\cdot}34 \times 10^3$ m/s. For each of the 20 subjects the average of the log of nail ultrasound velocity for all 10 nails was calculated; the range of velocity was $2{\cdot}03$–$2{\cdot}69 \times 10^3$ m/s (Fig. 5). Significant differences between subjects were demonstrated.

Derived thickness

The relationship of ultrasound derived nail thickness to finger position, and the almost constant differences between proximal and distal positions are shown in Figure 6. The transit times were converted to 'derived thickness' using the ultrasound velocity of $2{\cdot}26 \times 10^3$ m/s. The right thumb distal micrometer readings demonstrated that nails in the males ($0{\cdot}59$ mm $\pm 0{\cdot}09$) were thicker than those in the females ($0{\cdot}49$ mm $\pm 0{\cdot}07$) ($n = 10$, $P < 0{\cdot}001$, Wilcoxon signed ranks test).

Reproducibility of micrometer and ultrasound

The typical variability of measurement by micrometer and ultrasound is illustrated for one nail in Figure 7. The coefficients of variation for micrometer and ultrasound readings for each nail and for the three subjects are illustrated in Figure 8, and show that the ultrasound readings have a lower coefficient of variation than the micrometer readings.

The average coefficient of variation for ultrasound measurements was $4{\cdot}02\%$ (SD $\pm 1{\cdot}28\%$). The average coefficient of variation between the multiple (five) micrometer measurements taken from each nail was $4{\cdot}98\%$ (one sample period). Between the daily measurements (mean of five readings) the average coefficient of variation was $5{\cdot}29\%$ (SD $2{\cdot}36\%$).

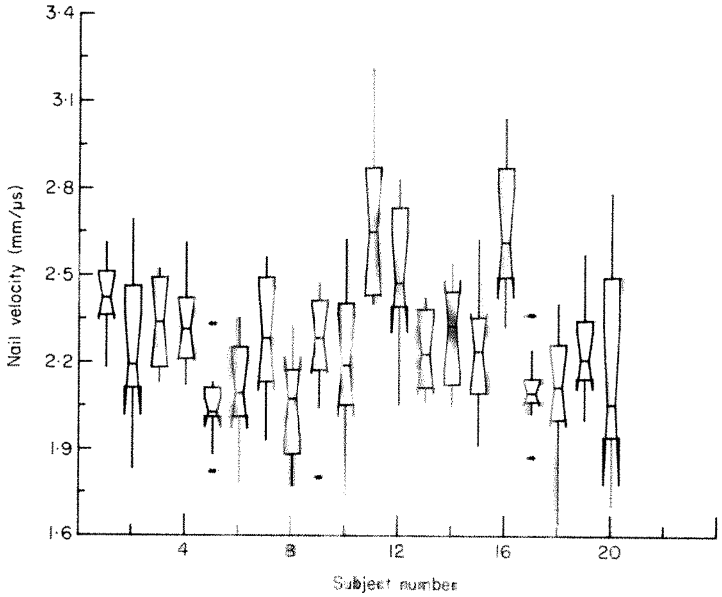

FIGURE 5. Notched box and whisker plot to show nail ultrasound velocity for each of 20 subjects.

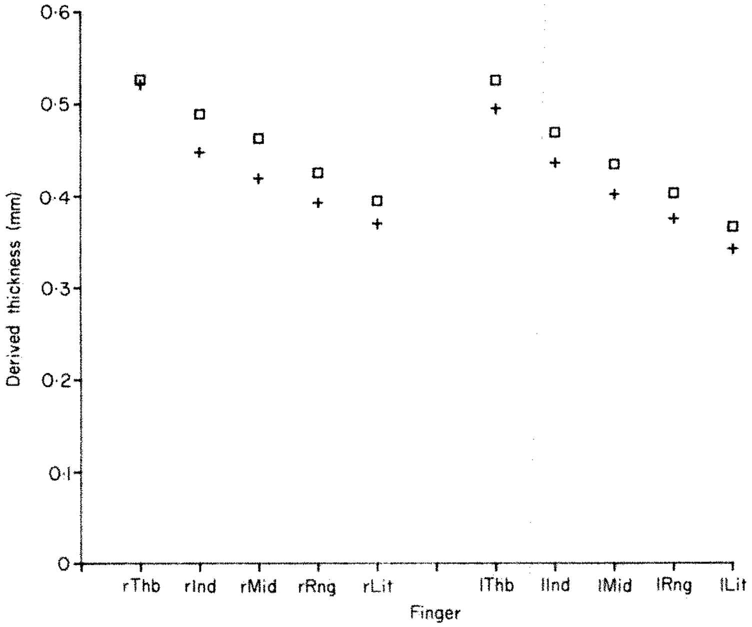

FIGURE 6. Derived ultrasound thickness for different finger positions for all 20 subjects, at the proximal and distal sites. +, Distal; □, proximal.

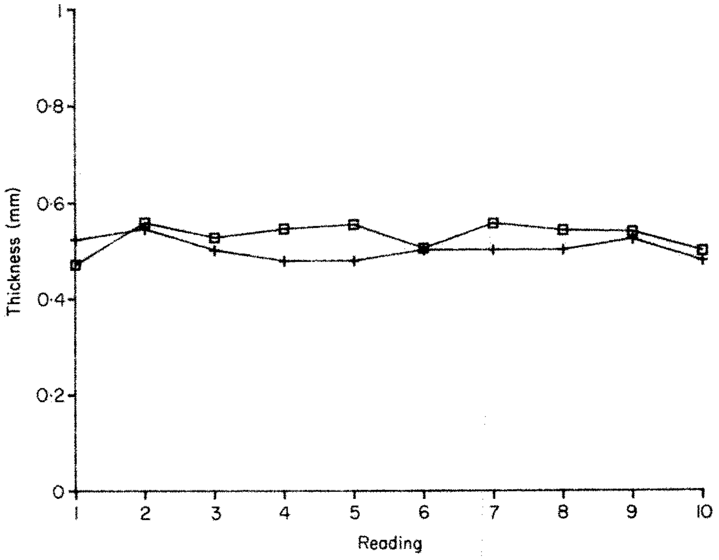

FIGURE 7. Daily readings in ultrasound derived thickness and micrometer thickness readings of the distal portion of one nail. +, A-scan; □, micrometer.

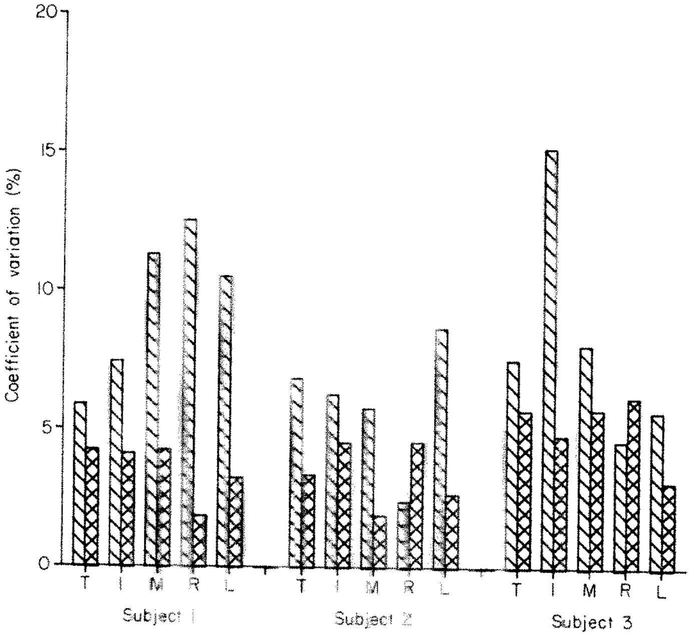

FIGURE 8. Coefficients of variation of ultrasound and micrometer readings for each nail studied on three subjects (subjects 1 and 2, 10 days; subject 3, 8 days). ▧, Micrometer; ⊠, A-Scan.

Effect of water immersion

The mean value of ultrasound transmission time in the 25 nails before water immersion was 0·20 ± 0·04 μs, and after 30 min of water immersion was 0·22 ± 0·04 μs ($P < 0{\cdot}01$, Wilcoxon). Within each pair the ultrasound transmission time either increased or remained constant, but never decreased. There was no difference between the mean micrometer measurements before (0·47 mm ± 0·08) and after (0·46 mm ± 0·09) water immersion. The derived ultrasound velocity before water immersion was 2·36 mm/μs (± 0·18) and after was 2·15 mm/μs (± 0·17) ($P < 0{\cdot}001$, Wilcoxon signed ranks test).

DISCUSSION

The present estimate of ultrasound nail velocity *in vivo* of $2{\cdot}26 \times 10^3$ m/s is within the same range of the estimate previously reported of 1600–2800 m/s.[1]

The decision over which echo points on the oscilloscope to measure is difficult. The initial large echo is a complex reflection of the membrane, outer surface of the nail and perhaps of the outermost layer of nail of a different density to the rest of the nail.[2] We agree with the general conclusions of Jemec and Serup[2] that density of nail plate varies through the nail, with the deeper nail being more hydrated.

It is also possible that nail water content differs across the nail plate in the longitudinal axis and it may be that the proximal nail has a higher water content (and hence lower ultrasound velocity) than the distal nail. This might explain the consistently increased ultrasound transit time (approximately 10%) of the proximal compared to the distal part of the nail. Until we have absolute methods of measuring proximal nail thickness or hydration *in vivo* the interpretation of the proximal readings must be viewed with caution, but we feel that hydration differences, if they exist, could not explain all the observed transit time changes between proximal and distal positions.

The observed significant differences in average nail velocity between some subjects suggests that there are differences in the structural composition of normal nails between individuals. These differences will have to be taken into account if such techniques are used in the measurement of nail thickness in nails affected by disease or drugs.

The micrometer and ultrasound readings both have low variability. One practical problem with the micrometer is the exact siting of the 'gripping' anvil jaws; if they are pushed too far proximally there may be too large a reading because of inclusion of compressed adjacent underlying epidermis. The other problem inherent in the micrometer used is the small constant force of compression between the calipers of 8 N caused by a spring within the instrument. This may cause a variable compression of the nail, depending on the nail softness. This error would presumably be greater, i.e. the micrometer reading would under-read the true value, the softer or wetter the nail; this effect may have been operating in our study. To circumvent this problem, the area of the anvil points could be slightly increased to spread the caliper force, and reduce the compression of softer nails.

It was predicted that after water immersion, the softer and expanded nail plate would transmit sound less fast and this was the case.

The persisting fundamental problem is that we still have no absolute method for measuring proximal nail thickness *in vivo*. Until a method is developed we will still not be able to measure ultrasound velocity at different sites in the nail, and therefore not accurately relate ultrasound transit times to nail thickness. However the method and derived information described above represents the most accurate *in vivo* approach currently available. The method is particularly valuable for detecting changes at the same site over a period of time.

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The prophylactic use of cyclooxygenase inhibitors in recurrent herpes simplex infections

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SUMMARY

In an open study, continuous oral therapy with cyclooxygenase inhibitors (ibuprofen or indomethacin) reduced the incidence and frequency of recurrent eruptions in nine of 16 patients with herpes simplex virus infection. Retrospective analysis of seven cases indicated that natural killer cell enhancement assays might allow us to predict the clinical outcome of such treatment.

Recurrent herpes simplex virus (HSV) eruptions occur in almost one half of infected patients. The appearance of recurrent disease seems to be influenced by the immune state of the host and can accompany certain conditions such as fever, menses, trauma, stress and following exposure to ultraviolet light. These observations and the fact that immunocompromized patients are particularly susceptible to these episodes have led to the suggestion that immunosuppression may be involved in the recrudescence of active disease.

The emergence of an increased proportion of suppressor T cells in the peripheral blood has been demonstrated at the time of recurrent disease.[1] These peripheral mononuclear cells (PMC) inhibit the HSV-induced proliferation of autologous PMC *in vitro* and elaborate suppressor factors when exposed to HSV proteins. The production of these monokines was prevented by the addition of cyclooxygenase inhibitors to the culture media.[2,3] Prostaglandin (PG) treatment increased virus replication *in vitro*[4] and PG inhibitors counteracted this effect. Events known to produce a local or systemic increase in PG production are frequently regarded as triggers for recurrent disease. PG interferes with host-defense mechanisms against HSV, including the production and activity of interferons (IFN) and the antibody-dependent cell-mediated cytotoxic activity of mononuclear leucocytes.[4] Based on these findings it was considered that cyclooxygenase-inhibiting drugs might be effective in the prophylaxis of recurrent HSV disease.[2,4] There has been only one report of treatment of recurrent herpes genitalis by a PG

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inhibitor[3] in which patients were treated with ibuprofen 600 mg three times a day for 5 days beginning with the first sign of the appearance of recurrent clinical disease.[3] There were no significant differences between the placebo and treatment groups. However, this might have been suboptimal treatment and to be most effective therapy must be initiated before the appearance of symptoms. PG inhibitors would interfere with the generation of the suppressor T lymphocytes associated with the onset of recrudescent lesions and should be considered for continuous therapy.

METHODS

Patients

Sixteen adult, healthy out-patients with recurrent HSV infections seen between 1984 and 1987 were enrolled in the study. All patients were diagnosed clinically and all but one had recurrent disease for at least 1 year and as long as 8 years (Table 1). Serology was performed on entry into the study using the multiplicity analysis kinetics of neutralization assay that specifically differentiates between antibody to HSV types 1 and 2.[5] Virus was isolated from the lesions and typed by immunofluorescent staining with HSV type specific monoclonal antibody (Syva Microtrak, Palo Alto, CA, U.S.A.) as previously described.[6] No patient received immunosuppressive or antiviral therapy during the course of the study or in the previous 6 months. All patients were examined every 2–3 months for at least 1 year after being included in the trial. The patients were treated orally with either indomethacin 25 mg or ibuprofen 400 mg four times daily, depending upon their ability to tolerate the gastrointestinal irritation. Six patients remained on indomethacin for the duration of the study, four patients were transferred within a month from indomethacin to ibuprofen and the remaining six were switched from indomethacin to ibuprofen after 5–7 months of therapy.

TABLE 1. Clinical effect of cyclooxygenase inhibitors against recurrent HSV

Patient (No/age/sex)	Location	HSV type (culture or or serology)	Associations	Duration of therapy (months)	Frequency (days) Recurrence before drug	Frequency (days) Recurrence on drugs	Years of disease prior to treatment
1/30/F	Facial/thigh	1&2		12	21	No change	1·5
2/40/F	Buttock/leg	1&2	Stress/trauma	14	30	No change	1
3/37/F	Genital	2	Menses	12	14	No change	3
4/33/F	Genital	1&2		17·5	30	No change	1
5/33/F	Genital	1&2	Infections	7	30	No change	1/3
6/21/F	Genital/buttock	1&2		13	30	No change	1
7/48/M	Genital	2		12	30	No change	4
8/70/F	Leg	1		12	30	120	3
9/37/F	Genital	1	Infection/trauma	24	60	243	2
10/30/M	Lip	1		13	30	90	1
11/50/M	Nose	2		42	15	365	5–6
12/38/M	Facial	1		24	60	485	2
12/30/M	Genital	2	Stress/drug/allergy	12	30	365	2
14/39/M	Genital	1&2	Stress	36	45	305	8
15/46/M	Buttock	2	Stress/trauma	12	30	90	3
16/35/M	Genital/lip	2		36	37	70	1·5

Cells and virus

Human epidermoid carcinoma No. 2 (HEp-2) cells were grown in medium 199 with 10% calf serum. They were used for the growth of the G strain of HSV-2 and for antigen preparation. Virus isolation was done on MRC-5 cells (human lung fibroblasts) obtained from M.A. Bioproducts, Walkersville, MD, U.S.A.

Antigens

Antigen was prepared as previously described.[1] HEp-2 cells 'mock' infected with phosphate-buffered saline (PBS) or with HSV-2 for 24 h were lysed by freezing and thawing and cleared of cell debris by centrifugation at 3000 ***g*** for 10 min. Virus was pelleted by centrifugation at 113,000 ***g*** for 1 h and resuspended in PBS to a final concentration of 200 μg protein/ml and inactivated by exposure to ultraviolet (UV) irradiation at 17 cm from a Sylvania G15T8 light source for 30 min. Antigens were used at a final concentration of 10 μg protein/ml.

Lymphocyte culture and supernatant collection

Peripheral mononuclear cells were isolated from seven randomly selected patients by density gradient centrifugation on Ficoll-metrizoate gradients from 40 ml of heparinized (20 U/ml; Upjohn Co; Kalamazoo, MI, U.S.A.) venous blood as previously described.[1] Viability as determined by trypan blue dye exclusion was $>95\%$. Cells ($2{\cdot}5 \times 10^6$) were cultured at 37°C in RPMI 1640 with 100 U/ml penicillin G, 100 μg/ml streptomycin, 25 mM HEPES buffer (Fisher Scientific Co., Pittsburgh, PA, U.S.A.) and 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY, U.S.A.). Cultures (1 ml) were set up in snap-cap tubes (12 × 75 mm, Falcon 2054, Oxnard, CA, U.S.A.) in the presence of antigen with or without 2×10^{-6} M indomethacin (Sigma Chemical Co, St Louis, MO, U.S.A.) and incubated at 37°C for 24 h. At this time the cells were pelleted by centrifugation at 300 ***g*** for 10 min and the supernatants were collected and stored at −70°C until assayed for natural killer (NK) cell-enhancing activity.[1]

NK enhancement assay

Peripheral mononuclear cells obtained from normal healthy volunteers 20–34 years old were used as effector cells. K562 erythroleukaemia cells were used as target cells. They were maintained in a stationary suspension culture and passaged 1 day before assay. K562 cells (5–20×10^6) were labelled for 1 h with 100 μCi of ^{51}Cr (NaCrO$_4$; New England Nuclear, Boston, MA, U.S.A.) at 37°C, washed three times and resuspended in RPMI 1640 with 10% heat-inactivated FCS. Triplicate samples of NK effector cells (4×10^5 cells/microtitre well) were incubated for 2 h at 37°C in 50 μl of the supernatants obtained from the antigen-stimulated PMC cultures. ^{51}Cr-labelled K562 cells were added directly to the wells of conical-bottom microtitre plates (Dynatech, Alexandria, VA, U.S.A.) to yield an effector-to-target cell ratio of 40:1. The plates were incubated at 37°C for 4 h, and the supernatants were harvested and counted. Each plate contained an interferon (IFN) control consisting of NK effector cells incubated in 50 U of human IFNα instead of culture supernatant. ^{51}Cr-labelled K562 cells incubated with culture supernatants or 50 U of IFN in the absence of effector cells served as a negative control. Radioactivity values in the negative controls were similar to the spontaneous release values. The enhancement of NK activity was calculated according to the following formula:

$$\text{NK enhancement (\%)} = 1 - \frac{\text{\% Specific lysis effectors}}{\text{\% Specific lysis effectors} + \text{supernatant}} \times 100.$$

Per cent specific lysis by effector cells averaged 24·2 ± 3 (SEM). The average percent NK-enhancing activity by effector cells exposed to IFN was 40 ± 5.[1]

RESULTS

All 16 patients studied in these series were HSV positive as determined by serology and/or virus culture. Six patients were positive only for HSV-2. Six were positive for both HSV-1 and HSV-2 and four were positive only for HSV-1 (Table 1). In eight cases the lesions were genital, in five cases they were facial and lesions on the leg, thigh or buttocks were seen in five cases. Nine of 16 patients (50%) experienced a significant decrease in the frequency and severity of their recurrent HSV disease over the course of the study and a dramatic improvement was seen in six of the nine. Males had a much higher response rate than females (88%: 25%, $P > 0·01$). There was no significant association between the HSV type or the location of the lesions and the success of the treatment programme. Three of the four patients reporting stress or trauma as a precipitating factor responded to therapy. There was no association between the type of cyclooxygenase inhibitor used and the clinical response.

Natural killer cell enhancement assays were performed on seven randomly selected patients on entry into the trial. Of these, four were patients who responded to cyclooxygenase inhibition therapy. Blood was taken from these four patients during the reactive phase of the recurrent disease episode at entry into the trial. In all four, addition of indomethacin to the PMC culture restored NK enhancing activity (responders). Indomethacin did not restore the NK enhancing activity of the three patients that failed to respond to treatment (non-responders) (Table 2).

TABLE 2. NK enhancement assays in patients who responded or did not respond to cyclooxygenase inhibitory therapy

	NK enhancement (%)	
Patient	HSV	HSV + indomethacin
Responders		
11	2 ± 1·3	30 ± 2
13	0	8 ± 1·3
14	20 ± 1	30 ± 2
16	6 ± 0·5	28 ± 1
Non-responders		
3	43·6 ± 8·3	34·7 ± 2·1
4	5·9 ± 5·0	0
5	0	0

HSV-herpes simplex virus. Values are listed with standard errors and the assays were performed in triplicate. Indomethacin was added to the culture assays at 10^{-6} M. All assays were performed on day 1 cultures. PMC were taken from patients during the recrudescence stage of the first disease episode at entry into the study.

DISCUSSION

Previous studies from this and other groups [1,5-9] have described the modulation of lymphokine responses related to the reactivation of latent HSV. It was shown that PMC obtained during active recurrent episodes contained a high proportion of suppressor T lymphocytes and failed to produce detectable levels of NK enhancing activity. PG contributes to the reduced activity, as normal responses were obtained when PMC were cultured with HSV antigen in the presence of indomethacin.[1,7]

Consistent with the interpretation that PG is involved in recurrent disease *in vivo*, we found that a proportion of patients with a relatively long history (1/3–8 years) of severe recurrent HSV disease and frequent exacerbation episodes (every 15–60 days) showed a significant decrease in the frequency of recurrent symptoms when receiving continuous oral cyclooxygenase-inhibitor therapy. The effect of the cyclooxygenase therapy was apparent within the first few weeks of treatment and there was no prompt rebound of clinical attacks when therapy was discontinued. The results of the NK enhancement assays in the seven cases studied indicated that NK enhancing activity might be able to predict the clinical result. Thus, PMC cultured in the presence of indomethacin were positive for NK enhancement activity in the responder patients, while the non-responders remained negative for NK enhancement activity.

The data must be interpreted with caution as the study group was limited to 16 patients and there was no control group. However, the beneficial effect of therapy was not likely to be due to a placebo effect or to improvement due to the length of time in the therapy protocol. Previous studies on similar patients with frequent attacks of genital herpes showed that 60 of 63 control patients receiving placebo noticed no change in the frequency of attacks.[10,11] There was a good correlation between response to therapy and restoration of NK-enhancing activity *in vitro*, and improvement in the responders was seen within a couple of months. The responders had a much longer history of recurrent disease (3 years) at entry into the study than the non-responders (1·7 years), which is consistent with the potential development of PG-dependent immunoregulatory controls of recurrent disease as a function of time after primary infection.

It is of interest that males responded much better to the cyclooxygenase inhibition therapy than females. Female sex hormones can influence the immune system and oestrogens appear to stimulate increased antibody production, whereas progesterone interferes with T-cell function.[12] A flare-up of HSV can be associated with menstruation and could be triggered by alterations in circulatory PG and/or progesterone levels. Whether cyclooxygenase inhibitors can modify the T-cell suppression induced by progesterone, is unkown.

Our studies demonstrate the efficacy of cyclooxygenase-inhibitor therapy for recurrent HSV infections and indicate the need for a larger controlled study including whether there is a possible synergistic activity of cyclooxygenase inhibitors with acyclovir.

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Effects of haem arginate on variegate porphyria

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SUMMARY

Four patients with variegate porphyria (VP) were treated with repeated haem arginate infusions daily for 4 days and then weekly for 4 weeks. After the initial four daily doses of haem arginate (haem 3 mg/kg), the excretion of faecal protoporphyrin (mean 579 nmol/g dry wt) fell to an almost normal level (mean 123 nmol/g dry wt), and that of coproporphyrin (mean 162 nmol/g dry wt) to the normal level (mean 21 nmol/g dry wt) in all patients. However, during the period of the four weekly infusions of haem the excretion of porphyrins increased almost to the pretreatment level. Phototesting showed no changes in the photoreactivity of the skin, and no improvement in skin lesions was seen during the treatment. Except for one case of thrombophlebitis no side-effects occurred.

In a child with homozygous VP, four daily infusions of haem arginate (2 mg/kg) normalized the faecal protoporphyrin content, but had no effect on the increased erythrocyte protoporphyrin concentration.

Variegate porphyria (VP) is characterized clinically by acute systemic symptoms and skin symptoms.[1] The biochemical diagnosis of VP is based on the increased excretion of porphyrins, predominantly protoporphyrin, in the faeces. The genetic defect in the haem biosynthetic pathway is in protoporphyrinogen oxidase which shows decreased activity. The skin symptoms are manifested as erosions and blisters of sun-exposed skin, a consequence of abnormal skin fragility. Slow healing and scarring of the lesions are characteristic features. These cutaneous findings are identical to those seen in porphyria cutanea tarda (PCT).

Haem arginate is a new haem preparation used for the treatment of acute porphyric symptoms.[2,3] Administration of haem suppresses the rate-limiting enzyme of the haem biosynthesis, delta-aminolaevulinic acid synthase, by negative-feedback control and decreases the production of haem precursors.[4–6] The side-effects of haem arginate are rare,[3] in contrast to conventional haematin which affects coagulation factors and causes thrombophlebitic reactions at the site of infusion.[4]

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Although the preliminary results suggest that haem arginate corrects the overproduction of porphyrins in VP,[3,4] little is known about the effects of haem in VP, and the long-term effects of haem in hepatic porphyrias. Because no treatment for the skin symptoms of VP is known we investigated the effects of long-term haem administration on porphyrin metabolism and on the photoreactivity of the skin in four patients with VP, and the effects of a brief course of haem therapy in a patient with the rare homozygous VP.

METHODS

Subjects

Four female patients with VP, aged 19–44 years, were studied. The diagnosis of VP was based on typical skin symptoms, increased excretion of faecal porphyrins, low activity of lymphocyte protoporphyrinogen oxidase and characteristic histopathology of the skin.[7] All patients experienced erosions and two of them also had blisters on the backs of the hands as a result of skin fragility. Three patients had mild fragility of the facial skin. The symptoms worsened in late summer and autumn. Besides the skin symptoms typical of VP, one patient had solar urticaria in early summer. No acute systemic symptoms occurred at the time of the study.

An 8-year-old boy with homozygous VP was included in the study. The patient had had severe skin symptoms since infancy.[8] In summer the patient had blisters and extensive erosions in the sun-exposed areas.

Haem administration

Infusions of haem arginate (Normosang®, Leiras Pharmaceuticals, Turku, Finland) were initially given four times on consecutive days, and then once a week for 4 weeks. Each dose was 3 mg haem/kg body weight. Ampoules of haem arginate were diluted in 100 ml physiological saline and given intravenously into a peripheral vein over 15 min. The treatment was carried out in the late spring for two patients and in autumn for two patients. The patient with homozygous VP was treated by haem arginate 2 mg/kg, for 4 consecutive days.

Laboratory investigations

Faecal coproporphyrin and protoporphyrin were determined according to the method of Rimington.[9] The mean of the values of three samples before the treatment was used as a reference value. After haem treatment had been started faecal samples were taken daily for the first 10 days, and then twice a week (3–4 and 6–7 days after the previous infusion) up to 3 weeks after the last infusion.

Blood samples were taken before the treatment, after the 4 daily infusions, before each weekly infusion and 1 week after the last infusion. The plasma protoporphyrin content was determined by liquid chromatography.[10] The level of plasma haemopexin (a haem-and porphyrin-binding protein)[11,12] was measured by immunodiffusion (Nor-Partigen, Hoechst). Blood haemoglobin, leucocyte and platelet counts and serum iron, ferritin, transferrin, aspartate aminotransferase, alanine aminotransferase and glutamyltransferase levels were routinely measured.

In the patient with homozygous VP, the excretion of faecal porphyrins, and erythrocyte and plasma protoporphyrin concentrations as well as the plasma haemopexin concentration were estimated before the treatment, and then daily during the infusions. Urine samples for measurement of uroporphyrin and coproporphyrin were collected before and during the treatment, at 24-h intervals. Faecal, urinary and blood porphyrin levels were determined by liquid chromatography.

Phototesting

A metal halogen lamp (Epolux, 2000 W, Airam, Finland) was used as the light source in phototesting. The lamp emitted radiation at 390–720 nm, peaking at 400–420 nm. The irradiance of the lamp at 400–410 nm varied between 21–53 W/m^2 as measured by spectrophotometry (Photo Research PR-703) at a distance of 15 cm, the distance used for testing. The phototesting was always performed on the same skin area on the volar side of the forearm and each irradiated area being about 3 cm^2. The exposure times were graded in periods of 5 min. The test was carried out before the treatment, after the four daily infusions of haem, after the second weekly infusion and 1 week after the last infusion. The shortest time of irradiation capable of eliciting erythema was taken as the threshold dose. To exclude initial heat erythema, the reactions were recorded 1 h after irradiation. In four control subjects the erythema threshold level (without haem infusions) was determined by the same method.

Clinical examination

The skin symptoms were examined by the same investigator before the treatment and later at the time of each phototesting. To assess the severity of skin symptoms the new and old lesions were counted.

RESULTS

Biochemical effects

The mean pretreatment value for faecal protoporphyrin was 579 nmol/g dry weight (range 310–954, normal < 100), and that for faecal coproporphyrin was 162 nmol/g dry weight (range 104–251, normal < 30). After the initial four daily infusions of haem arginate, the excretion of protoporphyrin and coproporphyrin fell sharply (Figs 1 and 2). The lowest mean values both for

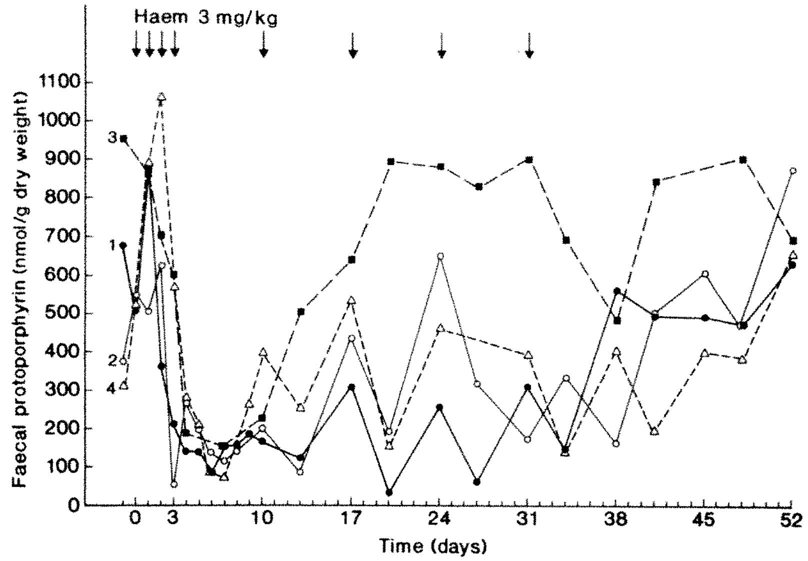

FIGURE 1. Effects of repeated haem infusions on the excretion of faecal protoporphyrin in four subjects with variegate porphyria.

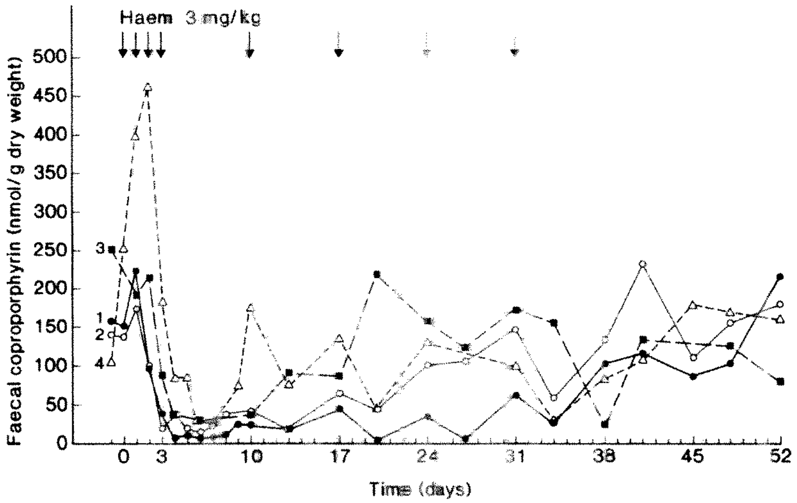

FIGURE 2. Effects of repeated haem infusions on the excretion of faecal coproporphyrin in four subjects with variegate porphyria.

protoporphyrin (123 nmol/g dry weight, range 72–153) and for coproporphyrin (21 nmol/g dry weight, range 7–30) were reached on days 7 and 8, i.e. three to four days after the fourth infusion. The difference between these lowest values and the pretreatment values was statistically significant ($P < 0·05$ for protoporhyrin, and $P < 0·02$ for coproporphyrin, paired *t*-test). Before the first weekly infusion of haem, the excretions showed, on average, a twofold increase from the lowest values. During the period of weekly infusions, the excretion of faecal protoporphyrin initially fell in three patients but returned within a week to almost the pretreatment level (Fig. 1). In one patient the weekly infusions had no effect on protoporphyrin excretion. The excretion of coproporphyrin showed a trend similar to that of protoporphyrin during the weekly infusions (Fig. 2). After the treatment, the excretion of faecal porphyrins remained high in all patients. Three weeks after the last infusion of haem, the mean faecal protoporphyrin level was 715 and that of coproporphyrin was 168 nmol/g dry weight, i.e. at the level of the pretreatment values.

The mean pretreatment value for the plasma protoporphyrin concentration was 10·4 nmol/l (range 4·7–18, normal < 3). After four daily infusions of haem, the mean plasma protoporphyrin concentration was 3·1 nmol/l (range 1·1–5·4; $P < 0·04$, paired *t*-test). During the weekly infusions, the values increased to the pretreatment level (Fig. 3). The mean pretreatment plasma haemopexin concentration was 0·731 g/l (range 0·425–1·015). It fell to zero after the four daily infusions of haem. During the weekly infusions, the plasma haemopexin concentrations were about 30% lower than before the infusions ($P < 0·04$, paired *t*-test).

The routine haematological measurements, the liver enzyme, serum iron and ferritin values showed no significant changes during the treatment (data not shown).

Clinical effects

In four healthy control subjects the irradiation time for the threshold of erythema was 60 min or more. In the patients, before treatment, this time varied from 5 min (the patient with solar urticaria) to 45 min. In one patient the irradiation time for erythema increased from 25 to 40 min during treatment, but in the other patients no changes in photoreactivity were observed. Similarly, no consistent changes in the skin symptoms were found during treatment. The skin

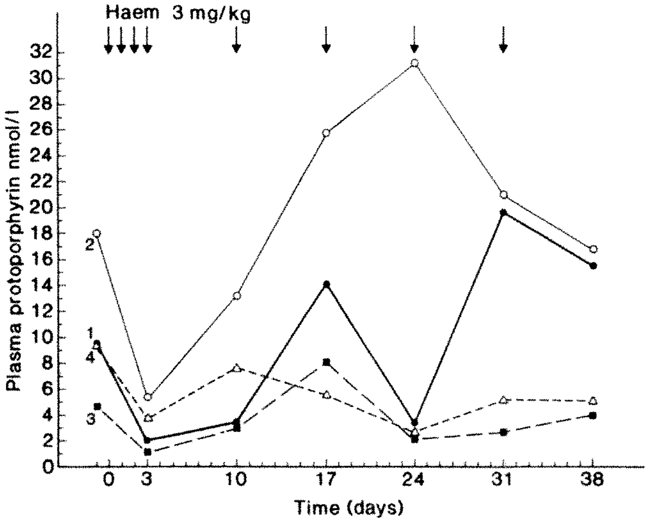

FIGURE 3. Effect of repeated haem infusions on the plasma protoporphyrin concentration in four subjects with variegate porphyria.

lesions disappeared in one patient but in the others the skin symptoms showed no response. One patient, in whom the photoreactivity diminished during treatment, had more skin lesions after the last infusion than at the beginning of treatment.

Side-effects

In all, 32 haem infusions were given, and only one mild thrombophlebitis occurred. There were no other side-effects.

Effects of haem in homozygous VP

In the patient with homozygous VP the mean pretreatment value for faecal protoporphyrin was 210 nmol/g dry weight (range of three determinations 106–374), and that for coproporphyrin was 36 nmol/g dry weight (range 17–51). After four infusions of haem, the faecal protoporphyrin value fell to the normal level, but 1 week after treatment the protoporphyrin excretion was increased to the pretreatment level at 190 nmol/g dry weight (Fig. 4). A similar initial decrease in the faecal coproporphyrin level was found. The excretions of urinary uroporphyrin (16 nmol/24 h) and coproporphyrin (119 nmol/24 h) were normal before the treatment, and no changes were found during the infusions. The pretreatment plasma protoporphyrin concentration was 141 nmol/l, and the lowest value was 33 nmol/l during treatment. The erythrocyte protoporphyrin concentration was 6960 nmol/l (normal < 1050) and remained at this level during the treatment. The plasma haemopexin concentration (0·275 g/l) decreased to zero during haem infusions. No side-effects occurred during treatment.

DISCUSSION

Our results showed that daily haem infusions effectively produced biochemical remission in VP. A 4-day course virtually normalized the faecal excretion of porphyrins and the concentration of plasma protoporphyrin. However, this effect was transient, as the values increased within a week. The weekly infusions did not maintain the faecal porphyrin content or the plasma

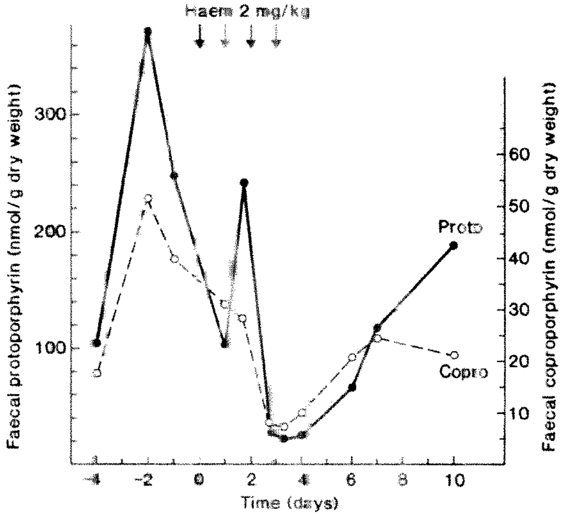

FIGURE 4. Effect of four haem infusions on the excretion of faecal protoporphyrin and coproporphyrin in a child with homozygous variegate porphyria.

protoporphyrin concentration at a low level. These results suggest that more frequent infusions of haem arginate, possibly twice a week, are required to obtain long-lasting biochemical remission.

The decrease of plasma haemopexin concentrations during treatment suggests that the kinetics of haem in VP is similar to AIP, and in the patients with myelodysplastic syndrome treated by weekly haem infusions.[4]

The treatment had no effect on the cutaneous symptoms of patients with VP or on the photoreactivity of the skin. Because skin symptoms in VP are of a chronic nature, it is not unexpected that a brief normalization of the plasma porphyrin concentration had no effect. A longer duration of treatment and more frequent haem infusions than used in the present study are probably needed for the skin symptoms to improve. Because no side-effects or changes in blood chemistry occurred in our patients, longer treatment periods might also be carried out safely. The study showed that infusions of haem arginate are easy to give to out-patients, although intravenous administration more frequently than once a week for several weeks may be impractical.

In porphyric subjects, photoreactions of the skin after irradiation at wavelengths of 400–410 nm (Soret band) are generally believed to be caused by increased amounts of porphyrins in the cutaneous tissue.[13,14] When plasma protoporphyrin concentration was lowest, the abnormal photoreactivity did not improve. This suggests that porphyrins in the skin are not in rapid equilibrium with plasma porphyrins, but are retained there despite temporary normalization of plasma protoporphyrin concentration. This supports the experiment by Meyer-Betz on himself who, after a single infusion of haematoporphyrin, experienced marked photosensitivity for several months.[15]

In the patient with homozygous VP four infusions of haem had a similar effect on the excretion of faecal porphyrins to the other subjects treated. The values normalized rapidly, but increased to the pretreatment levels within a week. The treatment had no effect on the high erythrocyte protoporphyrin concentration which is a characteristic feature in all hepatic homozygous porphyrias.[16] The laboratory method used did not differentiate between free and zinc-bound protoporphyrin, but excess erythrocyte protoporphyrin in homozygous VP is known to be mainly zinc-protoporphyrin.[17] The plasma protoporphyrin concentration in our patient was very high as compared with the patients with heterozygous VP. Haem administration only partially affected the plasma protoporphyrin level. This may have resulted from the diffusion of protoporphyrin from the erythrocytes to the plasma or from the inability of exogenous haem to suppress the overproduction of protoporphyrin by the liver in homozygous VP.

In conclusion, four daily infusions of haem arginate effectively normalized the faecal excretion of porphyrin and the plasma protoporphyrin concentration in heterozygous VP. Weekly infusions for 1 month were well tolerated, but did not achieve a satisfactory biochemical response and had no effect on skin symptoms or photoreactivity. In homozygous VP, the effect on the faecal porphyrin content was similar, but the response in plasma protoporphyrin concentration was only partial. Thus, the clinical value of haem arginate in VP is confined to the treatment of acute attacks.

ACKNOWLEDGMENTS

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Successful treatment of eruptive pyoderma gangrenosum with intravenous vancomycin and mezlocillin

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SUMMARY

A marked exacerbation of pyoderma gangrenosum in a patient with Crohn's disease was treated with intravenous vancomycin and mezlocillin and an excellent response was observed without any notable side-effects.

Pyoderma gangrenosum is an uncommon but well recognized ulcerative skin condition often associated with systemic disease. As in the initial series reported by Brunsting *et al.*,[1] ulcerative colitis continues to be the most frequently associated systemic illness,[2,3] but pyoderma gangrenosum has also been observed in patients with Crohn's disease,[4] chronic active hepatitis,[5] sero-positive[6,7] and sero-negative rheumatoid arthritis[6,8] and various haematologic abnormalities including acute lymphoblastic leukaemia,[9] multiple myeloma,[6] paraproteinaemia[10] and acute[11] and chronic myelogenous leukaemia.[12] In up to 50% of the patients, however, no associated systemic illness is present.[13,14]

Two clinical courses have been observed for pyoderma gangrenosum. In the acute type, pustules erupt and rapidly progress to ulcers that continue to enlarge unless arrested by treatment. A slower progression from pustules to ulcers that enlarge over weeks or months represents the other type.[15] Regardless of the clinical type, lesions of pyoderma gangrenosum usually heal spontaneously with characteristic atrophic, irregular and often cribriform scars.

Various treatments have been used for pyoderma gangrenosum but high-dose corticosteroids, in the form of oral prednisone, have been the mainstay of therapy for the acute eruptive type.[2,6] We report a case of eruptive pyoderma gangrenosum associated with exacerbation of Crohn's disease which was dramatically aborted with intravenous vancomycin and mezlocillin. We are not aware of any previous reports of pyoderma gangrenosum treated with these two antibiotics.

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CASE REPORT

A 46-year-old Caucasian male with large-bowel Crohn's disease diagnosed by colonoscopy presented to our department in July 1987 with multiple pustules and ulcers mainly on the trunk and with an associated fever. This episode was associated with a flare of his bowel disease. Based on the typical morphology of the lesions, the clinical setting, the negative wound cultures except for expected normal contaminants and the skin biopsy findings a diagnosis of pyoderma gangrenosum was made. The patient responded well to oral prednisone (60 mg per day) with the bowel becoming quiescent and with complete healing of all ulcers with characteristic cribriform scarring over 2 months. Because of intermittent exacerbations of the Crohn's disease and the patient's inability to tolerate sulphasalazine, oral prednisone was not finally tapered off until 10 months after the initiation of the therapy. The patient remained free of any disease activity for 4 months until October 1988 when his bowel disease became worse requiring 30 mg of prednisone per day. The bowel activity decreased transiently, but 3 weeks later, along with recurrent haemorrhagic diarrhoea, a tender pustule with 3 cm of surrounding erythema developed on the right upper shoulder and subsequently ulcerated. The dose of oral prednisone was doubled to 60 mg per day. Between 0·3 and 1 ml of triamcinolone (10 mg/ml) were injected almost weekly into the ulcer as well as into half a dozen tender sterile pustules and erosions that developed over the ensuing weeks. In addition, topical 2% sodium cromoglycate was applied three times a day to the ulcers. Despite this therapy, the ulcerated area remained unchanged and although injected pustules cleared, new pustules continued to appear. During this time, the bowel disease became progressively more acute. Azathioprine (50 mg per day orally) was also added in an effort to control both bowel and skin.

Despite this treatment, the patient suddenly appeared toxic with fever, chills and multiple tender 0·5–1 cm pustules on the scalp, chest, back, abdomen and scrotum (Fig. 1). Half a dozen shallow ulcers located on the shoulders and back that were being treated with intralesional

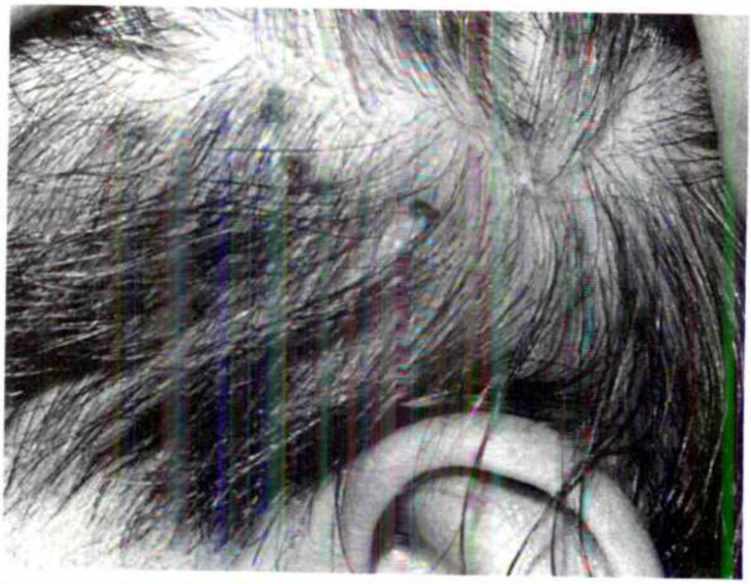

FIGURE 1. Grouped small pustules and shallow ulcers with surrounding erythema on the patient's scalp. These lesions were extremely tender to touch.

steroids and topical sodium cromoglycate increased in size, and developed surrounding erythema and discharge. Physical examination demonstrated no localizing signs of infection. The white blood count was normal. Gram stains of the discharge from multiple pustules showed only polymorphonuclear leucocytes without micro-organisms. Cultures from the pustules, blood, urine and stool were all negative.

Because of concern of infection 1 g of oxacillin was given intravenously and then intravenous vancomycin (1 g every 12 h) and mezlocillin (3 g every 4 h) were begun. Azathioprine was discontinued but prednisone was maintained at 65 mg per day. In less than 24 h after admission to hospital a remarkable improvement was noted and the patient became afebrile. Minocycline (100 mg every 12 h) was later added to the treatment regimen and the patient was discharged 10 days after admission to hospital with only the remnants of pustules and a healed ulcer on the shoulder. Since discharge, the patient has had repeated flares of his Crohn's disease and pyoderma gangrenosum but none severe enough to require further in-patient therapy. His disease activity is currently controlled with 30 mg of prednisone and minocycline 200 mg per day.

DISCUSSION

The pathogenesis of pyoderma gangrenosum is not clearly understood, despite numerous investigations into infectious, immunological and serological factors. With many seemingly unrelated systemic illnesses associated with the skin condition in nearly half of the cases, it is suggested that pyoderma gangrenosum represents a common cutaneous end-point of a number of different disease processes.[15] Numerous therapeutic modalities have been reported to be beneficial in pyoderma gangrenosum. Among topical therapies, 2% solution of sodium cromoglycate applied three times a day has been reported to be effective.[16] The use of topical corticosteroids has not generally yielded good results; however, intralesional injections of triamcinolone acetonide have sometimes caused a dramatic improvement.[17] Sulphasalazine was the first successfully and widely used systemic agent in the treatment of pyoderma gangrenosum.[18] Its active ingredient sulphapyridine, and sulphones, especially diaminodiphenylsulphone, have also shown good results,[19] and sulphones are considered by some to be the drug of choice for the chronic, slowly progressive form of pyoderma gangrenosum.[15] The efficacy of corticosteroids in pyoderma gangrenosum has been proven over the years and many consider them the drug of choice for the acute, rapidly progressive form of the disorder.[2,6] Sixty to 120 mg of prednisone are used initially to arrest the disease. More recently, intravenous pulse-therapy with methylprednisolone sodium succinate (1 g per day for 5 days) was reported to be beneficial in those who failed other more conventional treatments.[20] This form of therapy requires close patient monitoring as sudden electrolyte shifts may induce cardiac arrhythmias and subsequent cardiac arrests. Besides corticosteroids, many other immunosuppressive agents such as azathioprine, melphalan, cyclophosphamide and cyclosporin A have been used in the treatment of pyoderma gangrenosum, providing support for some immunological defect in the pathogenesis of pyoderma gangrenosum.[5,21-24] Clofazimine[25] and minocycline hydrochloride[26] are antimicrobial agents with reported therapeutic efficacy in the disorder. With minocycline, as with tetracycline, an anti-inflammatory property of the drug is thought to be at work. Although most of these drugs have shown their efficacy while treating the underlying systemic diseases, they have also been used successfully in cases not associated with an identifiable systemic disorder.[23,24]

Our patient presented with eruptive pyoderma gangrenosum without sepsis and associated with a worsening of his Crohn's disease. The observation that the skin disease parallels that of the underlying bowel disorder has repeatedly been made,[1,2,6,18] although exceptions have been seen as well.[27] Because our patient had been on prednisone for 20 of the past 24 months and azathioprine for several weeks, sepsis leading to embolic pustules was the prime concern. There was a dramatic and rapid response to therapy with intravenous vancomycin and mezlocillin, and more aggressive treatment with systemic corticosteroids was not initiated.

We are unaware of any previous report demonstrating a therapeutic success in the acute form of pyoderma gangrenosum with these two antibiotics, separately or in combination. Systemic antibiotics have been used in the past with variable results and are generally believed to be unhelpful. Rifampicin has been used to successfully treat refractory chronic pyoderma gangrenosum associated with acute lymphoblastic leukaemia.[9] As neither of the antibiotics used in our case is known to be anti-inflammatory, we can only speculate on their role in this case. Damaged colonic mucosa from the inflammatory bowel disease could be the portal through which bacterial or dietary antigens are absorbed and result in immunological and/or non-immunological reactions that involve the skin. The Schwartzman reaction, in which tissue damage occurs through direct toxicity of Gram-negative bacterial endotoxin, has been proposed as a possible mechanism linking the inflammatory bowel disease and pyoderma gangrenosum.[28] The detection of autoantibodies directed against the skin and intestinal antigens[29] and isolation of epidermolytic protease in the stools of patients with pyoderma gangrenosum[30] also link the skin and the bowel. The clearing of pyoderma gangrenosum after proctocolectomy[31] and through elimination of *Escherichia coli* by selective decontamination of the digestive tract[32] support these hypotheses. Action against Gram-negative and anaerobic micro-organisms by vancomycin and mezlocillin, especially the latter, may then explain why such dramatic improvement was observed in our case.

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Skin manifestations in congenital deficiency of leucocyte-adherence glycoproteins (CDLG)

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SUMMARY

In congenital deficiency of leucocyte-adherence glycoproteins (CDLG) there is an immunodeficiency with impaired leucocyte function and cutaneous and extracutaneous infections occur. In more than 30% of cases the condition has a fatal course.

We report the skin manifestations of three siblings with CDLG in which areas of skin necrosis occurred that resembled pyoderma gangrenosum.

The congenital deficiency of leucocyte-adherence glycoprotein (CDLG) was first described in 1975,[1] and during the last 15 years 31 cases have been reported.[2–14] The condition of CDLG is characterized by a deficiency of α-subunits of three leucocyte-adherence glycoproteins: CD11a designated LFA-1 (leucocyte function-associated antigen 1), CD11b (also designated MAC-1), CD11c (P150,95) and a deficiency of the β-subunit CD18. As a consequence, there are abnormal adherence, decreased chemotaxis and impaired aggregation of polymorphonuclear leucocytes (PMN) and monocytes, a reduced metabolic burst of PMN and abnormal B-cell differentiation. In contrast, the functions of the T cells are essentially normal.

Impaired wound healing, necrotic cutaneous abcesses, gingivitis, periodontitis, ulcerative stomatitis, pharyngitis, otitis media, pneumonia, peritonitis and septicaemia are features of this immunodeficiency disorder. Of 34 patients described, 13 have died. High white blood cell counts occurred during active infections and these may be above 20,000/mm^3.

We report the skin manifestations in three siblings with CDLG over a 20-year period of follow-up, and the immunological aspects of the disorder in these patients.

CASE REPORTS

Neither of the patients' parents, who were unrelated, showed any of the clinical manifestations of CDLG although they were the carriers for the disease. Of the children, three sons and one

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daughter were healthy and in two sons and one daughter the diagnosis of CDLG was made. The daughter with CDLG had a son who was unaffected. The immunological data on Case 3 and his parents have been previously reported.[14] The patients' polymorphonucleocytes, monocytes and lymphocytes were found to be deficient for CD11a, CD11b, CD11c and CD18, as determined by semi-quantitative immunofluorescence with a panel of monoclonal antibodies. The granulocytes from these patients expressed amounts of β-chain at levels of 10% of normal and its expression was not detectable on monocytes and T lymphocytes.

The clinical manifestations of the cases are summarized in Table 1.

Case 1

A male, born in 1949, had recurrent ulcerations of the skin from the first year of his life. These were diagnosed as pyoderma gangrenosum. In 1968 he developed an abcess of the jaw and a fulminant pneumonia. He died in 1969. It was during this last illness that his white cell count reached values as high as 65,000/mm^3. The leucocyte functions of this patient were not tested and there was no other record of skin manifestations.

Case 2

A female, born in 1951, developed recurrent ulcerations of the skin from the first year of her life. These were preceded by the appearance of erythematous nodules that ulcerated and were localized to the pubic region (Fig. 1a), abdominal wall, perianal region, legs, arms and on the buccal mucous membrane. Between 1961 and 1980 she had massive areas of necrosis of the skin, with large ulcerated areas (Fig. 1b). The edges of these lesions were raised and undermined and were surrounded by a violaceous rim. There was little pus formation. In 1976 nummular erythematous scaly plaques were present on both legs that had a psoriasiform appearance. These

TABLE 1. Clinical data of three siblings with CDLG

Case 1		Case 2		Case 3	
Age (years)	Clinical data	Age (years)	Clinical data	Age (years)	Clinical data
1–19	Skin ulcers	1–28	Skin ulcers	From first years of life	Gingivitis, stomatitis and tonsilitis
11–17	Pneumonia, gingivitis, otitis media and sinusitis	7–28	Systemic corticosteroids		
				7	Skin ulcers
		7	Laryngo-tracheo-bronchitis		
19	Jaw abcess and fulminant pneumonia			8	Pericarditis
		13–28	Recurrent stomatitis	9	Subperiostal abcess
		21	Uncomplicated delivery of a healthy boy	11	Pneumonia
		25	Psoriasiform dermatosis	20–22	Skin ulcers
		28	Large perianal abcess and septicaemia	26	Skin ulcers and psoriasiform dermatosis

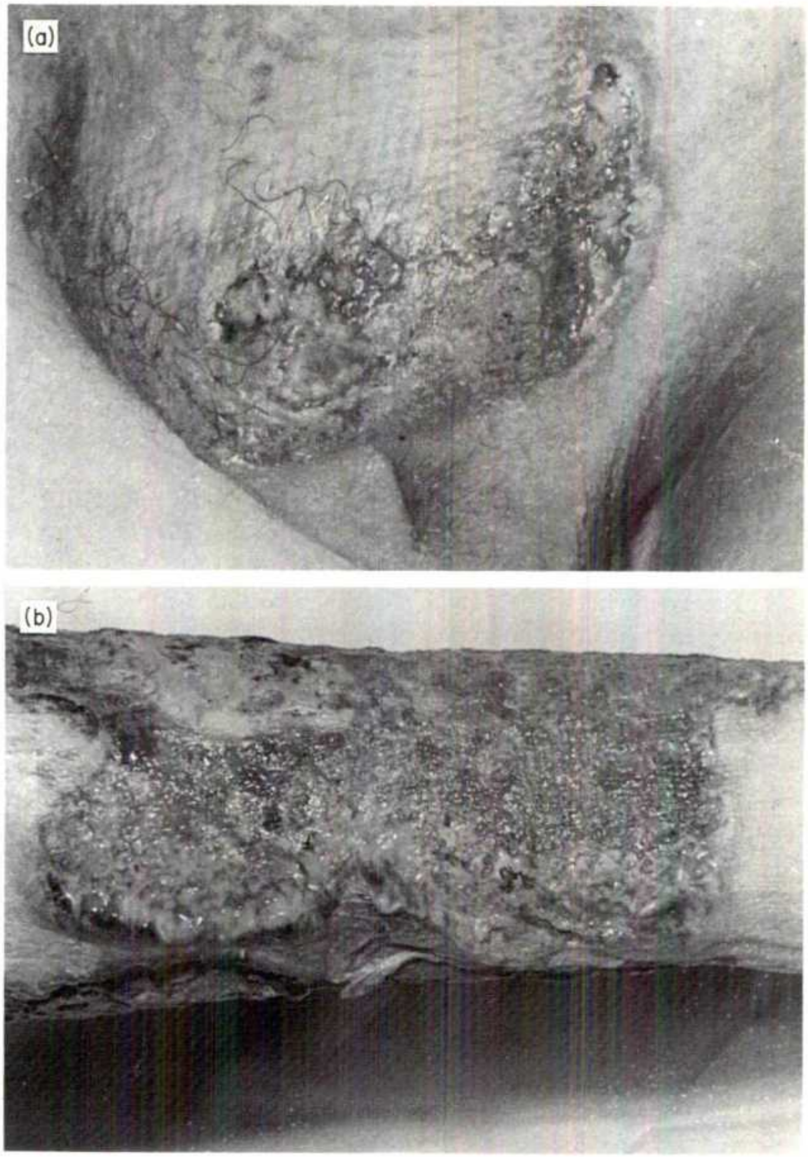

FIGURE 1. Case 2. (a) At the age of 7 years. Ulcers in the pubic area which are surrounded by a violaceous rim. (b) At the age of 23 years. A large ulcer on the lower leg. The edge is partly raised and has a violaceous colour.

lesions cleared following treatment with crude coal tar 1·5% and flumetason pivalate in an ointment base.

Treatment with systemic steroids was commenced in 1958 and the patient was on the equivalent of 20 mg prednisone per day as maintenance therapy. The areas of ulceration were extensive and painful and required repeated admissions to hospital. She was treated with various courses of antibiotics and different topical treatments. On admission to hospital the dose of systemic corticosteroids was increased to the equivalent 50–100 mg of prednisone. In 1980 a large perianal area of necrosis was complicated by her developing a septicaemia from which she died.

During the period when there was active ulceration of the skin, the WBC counts reached high values and during the last illness was as high as 100,000/mm^3. Cultures of swabs taken from the ulcers revealed *Staphyloccoccus aureus*, β-haemolytic *Streptococci* (group A), *Proteus mirabilis* and *Pseudomonas aeruginosa*.

A biopsy was taken in 1966 from an erythematous nodule in the pubic area. In the dermis there was a dense mixed inflammatory cell infiltrate that extended into the subcutaneous fat. At the margin of the biopsy, the infiltrate had a perivascular arrangement. In the central part of the biopsy, the epidermis was acanthotic and there were some areas in which there was an infiltrate of cells into the epidermis. The infiltrate consisted predominantly of PMN with some mononuclear cells. Plasma cells were also seen.

Case 3

A male, born in 1963, presented with skin manifestations of CDLG at the age of 7. Erythematous nodules and haemorrhagic blisters were present that developed into necrotic ulcers with a raised violaceous border (Fig. 2a). There was little pus formation. The lesions responded well to treatment with systemic antibiotics and various topical antibacterial treatments. Between 1983 and 1989 there were a number of relapses with areas of ulceration on the skin of the legs. These were treated with systemic antibiotics and local antibacterial preparations.

In 1989 he developed a number of non-itchy and sharply demarcated erythematous scaly plaques on the legs, upper arms, trunk, head and buttocks (Fig. 2b). Several scars where he had previous areas of skin necrosis were affected by these lesions. There was no abnormality of the nails. These plaques cleared following 3 weeks of treatment with hydrocortisone-17-butyrate in a cream base.

Swabs of the skin ulcers grew *S. aureus* and during the episodes of infection, the WBC counts ranged between 25,000 and 50,000/mm^3. The immunological investigations in this patient have been previously reported.[14]

A biopsy was taken from a scaly plaque on the right hip which showed acanthosis of the epidermis with elongation of the rete ridges and pronounced parakeratosis with intraepidermal collections of mononuclear cells. In the dermis the capillaries appeared dilated and tortuous. A perivascular infiltrate of mononuclear cells was also seen.

DISCUSSION

The clinical appearance of the skin ulcers in Cases 2 and 3 and the histopathology of the lesion biopsied in Case 2 were compatible with the diagnosis of pyoderma gangrenosum. Although pyoderma gangrenosum is considered to be a nosological entity, the condition occurs in association with a number of disorders including myeloproliferative diseases,[15,16] abnormalities in PMN function,[17,18] impaired cell-mediated immunity[19,20] and monoclonal gammopathy.[21,22] The patients described in this report have congenital deficiency of leucocyte-adherence glycoproteins (CDLG).

A feature of the pyoderma gangrenosum lesions in this family was the minimal amount of pus formation, and this reflects the impaired capacity of PMN and monocytes to invade into the tissues.[14,23] This is due to a defect in leucocyte-adherence glycoproteins CD11 and CD18 and is

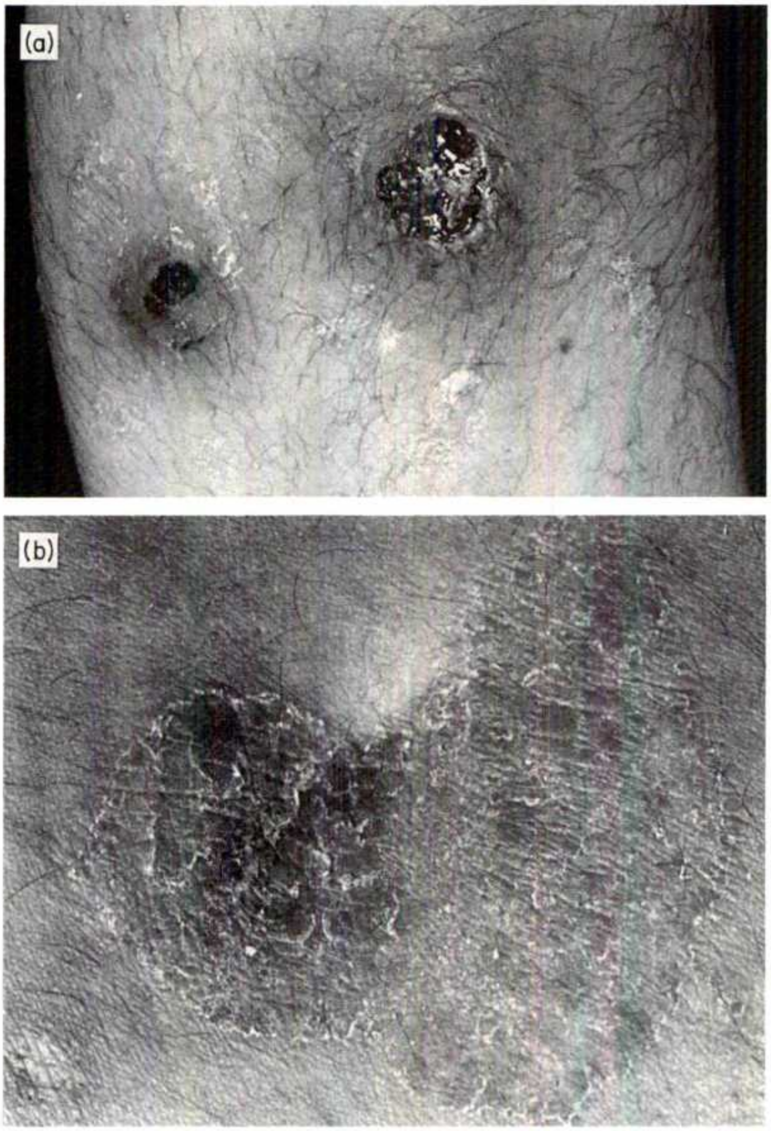

FIGURE 2. Case 3. (a) Ulcers with a violaceous rim, localized to the lower leg and surrounded by erythematous and scaly plaques. (b) Sharply demarcated plaque on the upper leg at the site of a scar.

a clinical feature of CDLG. In our patients and other cases of CDLG as reported in the literature, a marked leucocytosis has been observed during phases of active skin necrosis.[2-14] The decreased extravasation and margination of leucocytes during active infections results in accumulation in the peripheral blood of these cells.

The pyoderma gangrenosum in these patients usually responds well to treatment with systemic corticosteroids.[24-25] However, in Case 2 prolonged therapy with corticosteroids in dosages up to an equivalent of 100 mg prednisone did not prevent a fatal course. Case 3 was not treated with systemic corticosteroids and antibacterial therapy controlled the skin manifestations in this patient. Immunologically, there appears to be no rationale for the treatment of CDLG with systemic corticosteroids.

The occurrence of a psoriasiform eruption in Cases 2 and 3 is intriguing, and the erythematous scaly plaques seen had the appearance of those of psoriasis. However, no PMN were observed in the biopsy from an active lesion. Polymorphonuclear leucocytes are a feature of psoriasis and intraepidermal accumulations of PMN may show a focal distribution.[26] In the biopsy from Case 3 there was a complete lack of PMN in the infiltrate, which consisted of mononuclear cells and which showed epidermotropism. Such a picture can be seen in the initial stage of psoriasis[26] and it is possible that these patients had 'initial psoriasis' that could not develop into mature lesions because of an inability of the defective PMN to invade into the skin.

In conclusion, pyoderma gangrenosum should be regarded as a morphological diagnosis and patients with this disorder may have the condition of CDLG. This is particularly so when pyoderma gangrenosum is associated with sparse formation of pus, a high white-cell count, the occurrence of bacterial infections with a severe and sometimes fatal course in other members of the family. Treatment with antibacterial agents is indicated in these cases rather than systemic corticosteroids.

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An unusual case of acquired localized argyria

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SUMMARY

We report an unusual cause of localized cutaneous argyria, due to the cutaneous implantation of a silver earring back.

Localized cutaneous argyria is an uncommon condition but a well recognized occupational and medicinal hazard. It can be self induced. Rarely, localized argyria can be caused by the leaching of silver from jewellery and we report such a case.

CASE REPORT

A 17-year-old girl presented with a pigmented lesion behind her left ear following the recent press coverage about melanomas and other skin cancers. The lesion was not present at birth and was first noticed when she was 9 years old. It had slowly enlarged but was asymptomatic. She was known to be nickel-sensitive.

An 0·8 cm diameter blue–grey oval macule was present in the skin over the mastoid process of the temporal bone (Fig. 1). The lesion had a smooth surface and was firm and mobile but not tender. The blue–grey colour was uniformly distributed over the centre of the lesion and lightened towards the periphery. No cervical lymphadenopathy was palpable. This lesion was thought to be an ordinary blue naevus and it was excised.

The formalin-fixed excision biopsy sample could not be adequately bisected because of a hard central nodule, so a preliminary histology block was prepared from the softer outer zone. Paraffin sections stained with haematoxylin and eosin showed extensive deposition of small round regular granules of brown–black pigment, particularly concentrated at the epidermal basement membrane, along the basement membranes of eccrine sweat glands and ducts (Fig. 2), the sebaceous glands, and in the elastic lamina and basement membranes of large and small blood vessels. There was a less concentrated scattering of pigment throughout much of the dermis. The nature and distribution of the pigmentation was consistent with the diagnosis of localized cutaneous argyria. In order to confirm the diagnosis ultrathin sections were prepared for electron miscroscopy and electron probe microanalysis.

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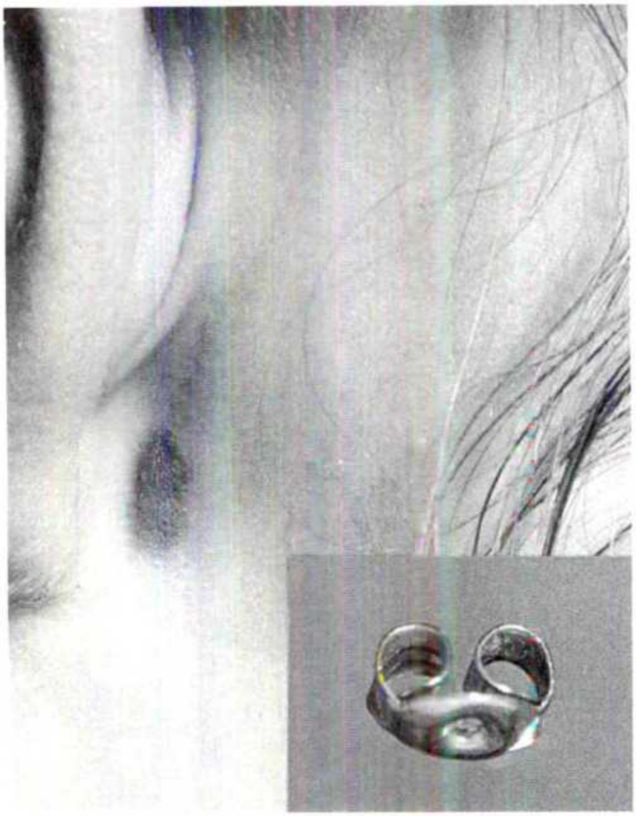

FIGURE 1. Blue-grey macule in the skin behind the left ear. Inset: silver earring back.

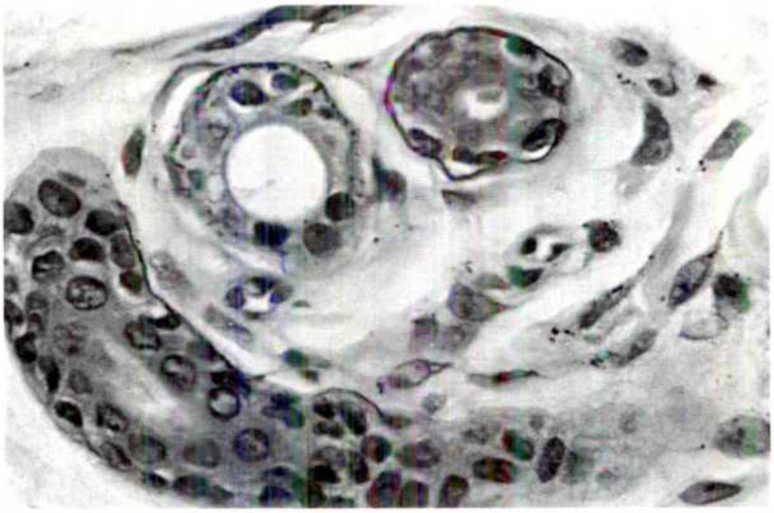

FIGURE 2. Part of an eccrine unit showing linear deposition of silver granules in the basement membrane. Note the scanty black granules in the cytoplasm of some adjacent connective tissue cells (haematoxylin and eosin, original magnification × 400).

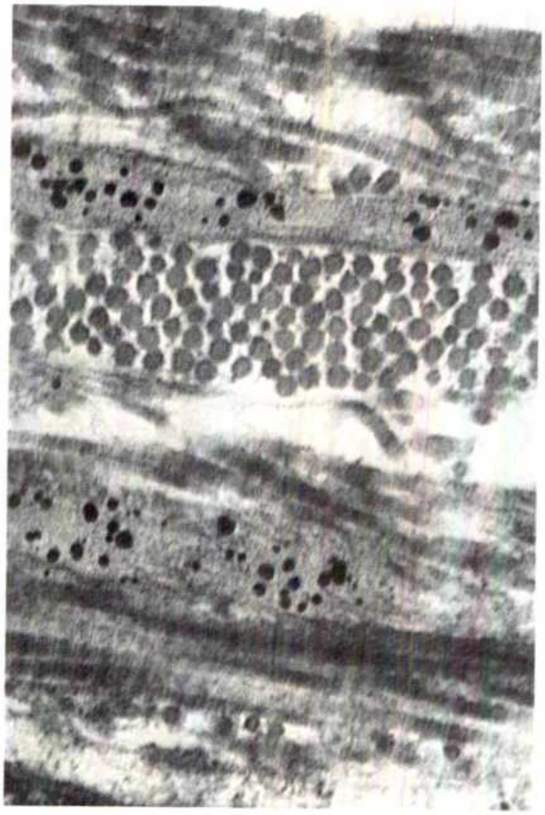

FIGURE 3. An electron micrograph showing silver granules deposited in elastin fibres rather than in the banded collagen (× 60,000).

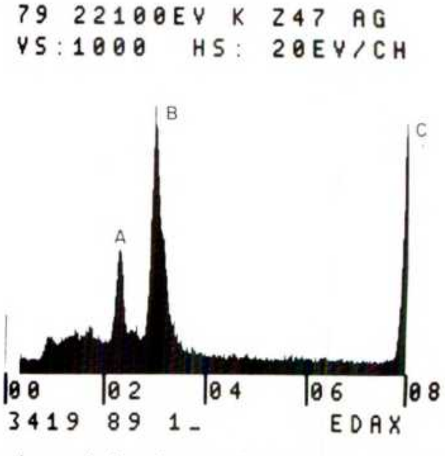

FIGURE 4. Electron probe microanalysis print-out. Peaks due to elements in electron-dense granules. A, sulphur; B, silver; C, copper.

Ultrastructural studies confirmed the presence of aggregates of electron-dense granules distributed along the basement membranes of the dermo-epidermal junction, eccrine and sebaceous glands. Between the collagen fibres in the reticular dermis dense spherical silver granules were demonstrated in elastic microfibrils (Fig. 3). The electron probe analysis confirmed that the electron-dense granules contained silver (Fig. 4). Two minor peaks, to copper and sulphur, were also recorded.

In view of the histology attention was turned to the remainder of the fixed specimen, and careful dissection revealed a metallic object embedded in a dense dermal fibrous nodule which proved to be a silver butterfly earring back (Fig. 1, inset). On further questioning it was discovered that she started wearing earrings when she was 9 years old. Her parents had bought her expensive, top quality silver earrings when they discovered that she was nickel-sensitive. As with many people who wear earrings, occasionally the backs went missing and she thought nothing of it. We surmise that, as a result of trauma, the silver butterfly back became embedded in the skin behind her ear, was corroded and released silver into the surrounding skin.

DISCUSSION

The commonest cause of argyria is mechanical impregnation of the skin by small silver particles in workers involved in silver mining, refining of silver, the manufacture of silverware and metal alloys, metallic films on glass and china, electroplating solutions and photographic processing. It has also been attributed to surgical and dental procedures such as silver amalgam-tattooing and silver sutures used in abdominal surgery. The prolonged use of topical remedies containing silver nitrate, such as eye drops, nasal drops, wound dressings and bladder irrigations, are another important cause. In Japan localized argyria is reported following the intracutaneous implantation of acupuncture needles.[1]

Irrespective of the route by which the silver gains access to the tissues, the histopathological and ultrastructural features are identical. Electron-dense silver particles were demonstrated along the basement membrane of eccrine sweat glands, dermo-epidermal junction and dermal elastic fibres and confirmed by X-ray microanalysis in biopsies from patients suffering from occupational argyria.[2] Identical findings were reported following the medicinal use of silver compounds[3] such as silver nitrate, applied topically, or given orally and following injection.

In this report we highlight a rare complication of pierced ears. As far as we are aware there is only one similar report in the English literature,[4] in which the localized argyria was confined to both ear lobes. The earrings had been removed from the patient's ear lobes 5 years prior to diagnosis although the discoloration was present at the time the studs were removed.

By law all British silverware must contain a minimum of 92·5% silver by weight. However, if the silver weighs less than one carat it does not have to be hallmarked. As silver is a very soft and pliable metal copper is added to the silver to strengthen jewellery (personal communication Dr K.Saunderson). This accounts for the three peaks visible on electron probe analysis. Silver was the largest peak as it forms the major constituent of the earring. The copper peak reflects both the copper grids and its addition to the earring. Most of the silver in the skin is deposited as silver sulphide, accounting for the peak for sulphur.

In a comprehensive study of ear piercing 34% of females with pierced ears complained of some local complications such as redness, swelling and infection.[5] Keloid formation, contact allergic dermatitis to nickel, bifid ear lobe deformity, staphylococcal septicaemia, sarcoid granulomas, cyst formation and organizing haematoma have all been described in association with ear piercing. Infected equipment has resulted in the transmission of hepatitis and a similar risk with HIV exists. The use of a spring-gun which fires a pre-sterilized gold plated stud through the ear lobe into a clip base has resulted in the stud occasionally being embedded in the ear lobe.

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Granulomatous cheilitis and Crohn's disease

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SUMMARY

A case of granulomatous cheilitis is reported in whom investigations showed intestinal involvement compatible with Crohn's disease, although the patient had no gastrointestinal symptoms.

Granulomatous cheilitis (GC) is an uncommon disorder that is characterized by persistent swelling of the lips and a granulomatous histology. Since the report of a case of GC that occurred several years before intestinal lesions appeared,[1] GC has been recognized to be an early manifestation of Crohn's disease. However, to our knowledge only four cases have been reported that initially presented with GC and then developed Crohn's disease.[1–4]

Whether GC represents an early manifestation of Crohn's disease or a distinct clinicopathological entity remains controversial.[5] We describe a patient with GC who had intestinal lesions compatible with the diagnosis of Crohn's disease, although she had no gastrointestinal symptoms.

CASE REPORT

A 56-year-old woman presented in September 1988 with a 2-month history of swelling of the left side of her lower lip. The swelling was persistent and did not fluctuate, but extended slowly around the mouth. There was no history of applied irritants or trauma. Apart from treatment for her hypertension she was otherwise in good health. Her weight was steady and she had no gastrointestinal symptoms. There was no relevant family history.

On examination, the left side of the lower lip was indurated, erythematous and swollen, and this involved the adjacent perioral skin (Fig. 1). The mucous membranes and gums were normal when first seen, but she had over the previous 2 years noted recurrent oral ulceration. There was no regional lymphadenopathy.

Blood investigations were normal, though she had an ESR of 34 mm/h. The serum iron was 57 μg/ml and the serum vitamin B_{12} was 1300 pq/ml. The serum angiotensin converting enzyme

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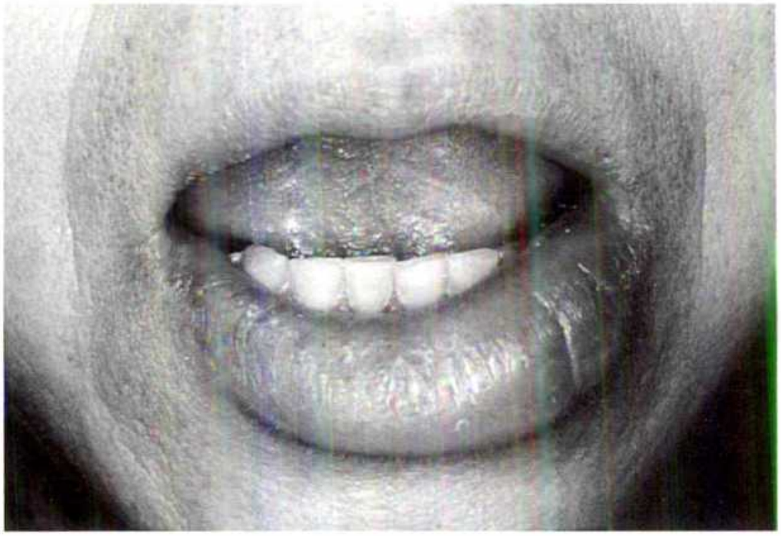

FIGURE 1. Persistent swelling of the left side of the lower lip.

level was within the normal range. Serological tests for syphilis and rheumatoid factor were negative. Tests for occult blood in the faeces were repeatedly negative. The chest X-ray was normal and her Mantoux test was positive. Because some of her teeth were crowned with metals, patch tests for metal allergens were performed as these had been previously described as possible precipitating factors.[6] The patch tests were all negative.

A labial biopsy showed multiple non-caseating granulomas throughout the dermis that extended to the underlying muscle. These granulomas were composed of lymphocytes, epithelioid cells and occasional multinucleated giant cells (Fig. 2). There were scattered plasma cells in the upper and mid dermis. No foreign bodies were seen when the sections were examined under polarized light. Stains for acid fast bacteria and for fungi revealed no organisms. A diagnosis of granulomatous cheilitis was made on the basis of the characteristic clinical and histopathological findings.

Although the patient had no gastrointestinal symptoms, sigmoidoscopy in addition to a barium enema, barium meal and follow-through were carried out. The sigmoidoscopy revealed that the patient had multiple ulcers involving the sigmoid colon and upper rectum. The remaining part of the colon and upper gastrointestinal tract appeared normal. A biopsy specimen was obtained from the recto-sigmoid colon and this showed non-caseating granulomatous inflammation suggestive of Crohn's disease.

The HLA antigens of the patient were A2, A24, Bw35, Bw54, Cw3, DR2, DRw53, DR4, DQw1 and DQw3.

DISCUSSION

A wide variety of skin manifestations have been described in Crohn's disease.[7-10] Labial swelling is well recognized although it is less common than oral ulceration. The association of GC with Crohn's disease is now well documented, and in four reports GC preceded the onset of Crohn's disease by several years.[1,3-5] Other patients have been described with Crohn's disease

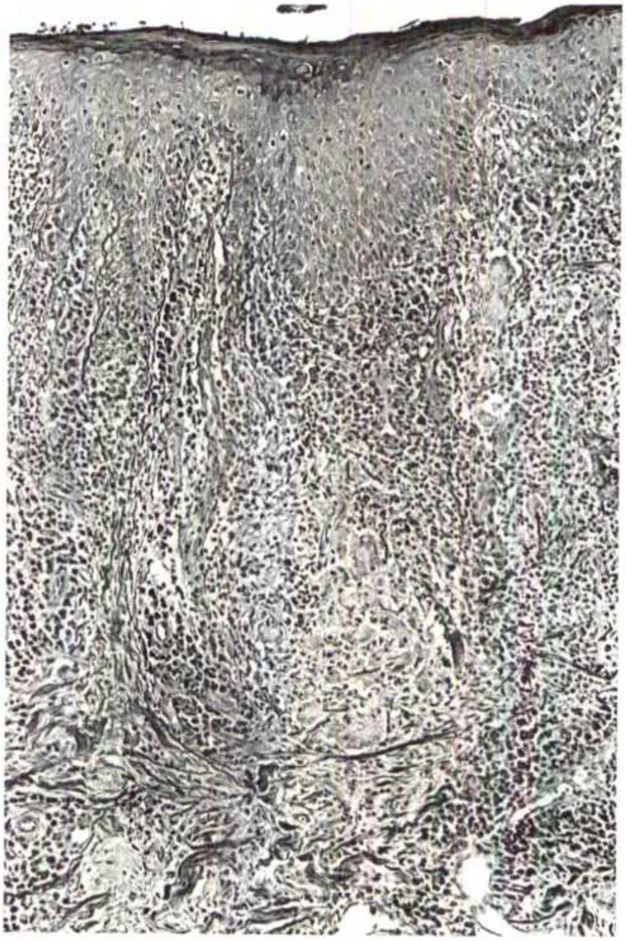

FIGURE 2. Biopsy of the lower lip showing an epithelioid cell granuloma with lymphocytes and multinucleated giant cells (H & E, × 100).

who later developed GC.[11-16] Tatnall *et al.*[16] described a patient with Crohn's disease who had metastatic cutaneous lesions and GC which they regarded as being a cutaneous manifestation of the disorder. Because of the paucity of reported cases of GC preceding Crohn's disease, it would be premature to conclude that this is an early manifestation of Crohn's disease, as was described by Carr.[1] It has been suggested that granulomatous cheilitis is a distinct entity, although it can be difficult to distinguish from labial Crohn's disease.[17] There is the possibility that intestinal lesions of Crohn's disease may develop later on. Our findings together with previous reports indicate the importance of a complete gastrointestinal investigation for Crohn's disease, even when the patient is asymptomatic. These patients require long term follow-up.

There has been some interest in the genetic predisposition to Crohn's disease, and one of us has studied HLA antigens in 47 Japanese patients with the disorder.[18] The class I antigen Bw61, and the class II antigens DR4, DRw9, DRw53 and DQw3 were found to be more frequent in patients with Crohn's disease when compared with 310 controls. Also, 16 cases in 12 families were examined to determine the HLA haplotypes associated with Crohn's disease and the haplotype of DRw53 and DQw3 found in 15 of the cases. This haplotype was found in our patient.

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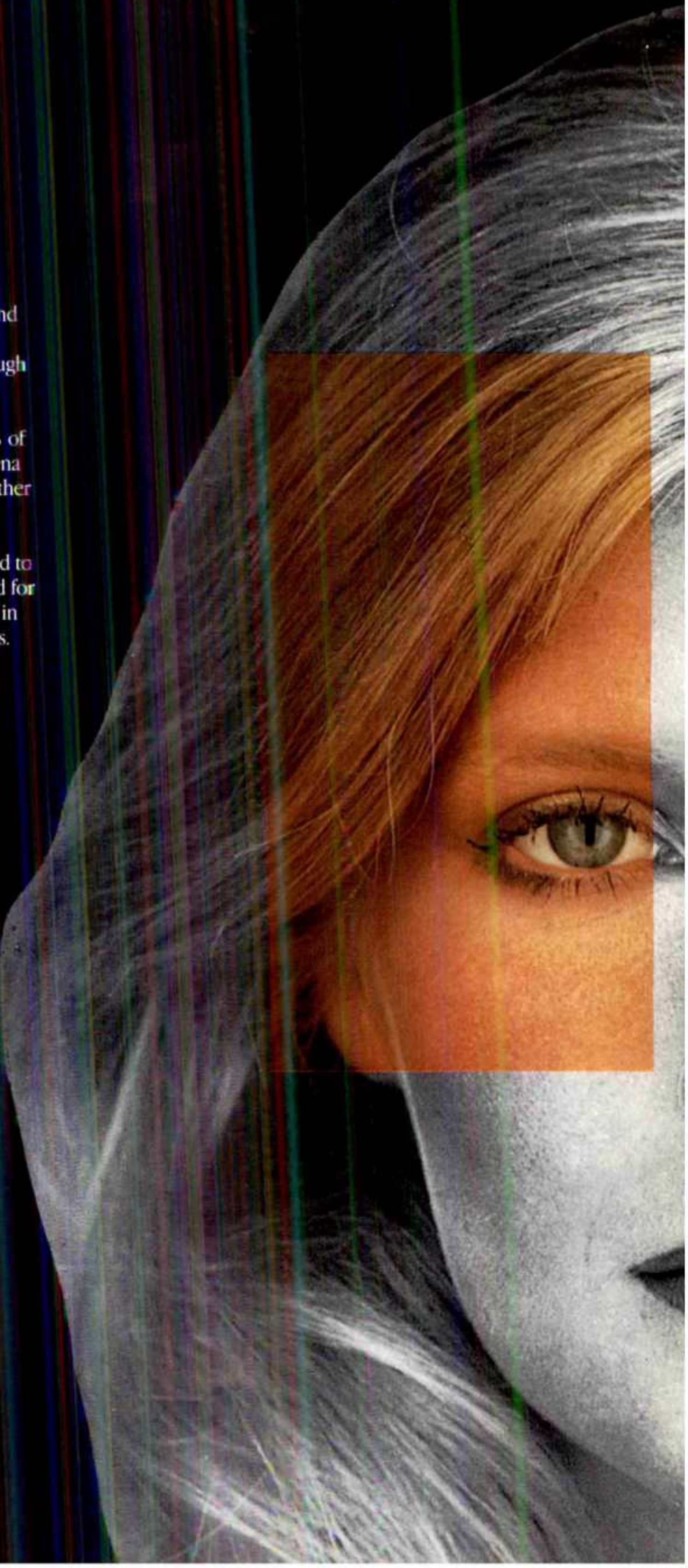

British Journal of Dermatology (1990) **123**, 413–416.

Correspondence

Kawasaki disease in a young adult with a perineal rash

SIR, In Kawasaki disease a perineal rash has been reported in children,[1] but not in adults. We report a case of Kawasaki disease in a young adult male with a perineal rash.

A 20-year-old man presented with a 5-day history of fever, headache, muscle pains and a sore throat. He later developed redness of his hands and feet. On admission to hospital he was pyrexial with a temperature of 40°C. He had an erythematous maculopapular eruption on the trunk and in the perineum, and painful erythema and oedema of the hands and feet. There was some conjunctival injection and dryness and erythema of the lips, a strawberry tongue and cervical and axillary lymphadenopathy. There were tender areas of erythema in the scrotum and perineum and this was more intense and separated from the erythema on the trunk and consisted of well-defined confluent maculopapular lesions. He had no itching and there were no vesicles or pustules. A few days later the rash became scaly.

Blood investigations revealed a white cell count of $12{\cdot}6 \times 10^9$/l with 65% polymorphonuclear cells. The platelet count was 240×10^9/l and 7 days later was 430×10^9/l. The ESR was 46 mm/h. Cultures of urine and blood were sterile, and a throat swab grew *Staphylococcus aureus* and α-haemolytic streptococci. Serological investigations for measles, Rickettsiae, Epstein–Barr virus and toxoplasmosis were all negative. The antistreptolysin titre was normal. The C3 and C4 fractions of complement and CH50 were normal. Rheumatoid factor and antinuclear antibodies were negative. Chest X-ray and ECG were normal and echocardiography showed evidence of a myocarditis but without any coronary artery involvement. Two days after admission to hospital he was treated with 3 g/day of salicylates and there was a reduction in his fever. He developed mild desquamation of the perineal region, followed by desquamation of the skin on the palms and soles. The echocardiography became normal 15 days after his admission.

The patient had all the major criteria of Kawasaki disease[2] and had other findings that are frequently associated with this disorder.[3] A differential diagnosis from toxic shock syndrome (TSS) was difficult,[3] because many of the symptoms and in particular the perineal eruption, are similar in both disorders. However, our patient had no systemic involvement and his fever persisted until salicylates were given, and he had no antibiotics.

Kawasaki disease in adults is rare. Our case is perhaps unique because of the perineal rash. This eruption in the perineal region resembled what has been previously described in children of tender or asymptomatic maculopapular and erythematous lesions separated from the macular erythema on the trunk and followed by scaling. Although this type of eruption can be observed in other disorders, the features of our case were more typical of Kawasaki disease. The prognosis in this condition depends on cardiac involvement, and especially if there are coronary artery aneurysms. An early diagnosis is important since arterial aneurysms can occur early in the disease.[4] Treatment with salicylates and/or with gamma globulins may prevent these aneurysms from occurring.[5]

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Aquagenic pruritus and the myelodysplastic syndrome

SIR, Aquagenic pruritus, water-induced itching without any visible skin changes, is a well-recognized condition that is usually of unknown origin. Some cases have been associated with haematological or lymphomatous disorders such as polycythaemia rubra vera, Hodgkin's disease and the hypereosinophilic syndrome. Aquagenic pruritus may precede underlying disease by several years. We report a case of a new haematological association of aquagenic pruritus and the myelodysplastic syndrome.

A 63-year-old civil engineer presented in December 1982 with a 6-month history of skin irritation after bathing. There was no previous history of atopy, urticaria or other skin disease. He had surgery for a ureteric calculus and also a prostatectomy, without any impairment of renal function. Examination revealed an otherwise healthy man who within 5–10 min of getting out of a bath containing water at any temperature, developed a generalized prickling sensation in his skin, particularly affecting the lower limbs. The symptoms lasted for up to 20 min. No visible skin changes were observed at any stage. He was not dermographic when tested with a spring loaded dermographometer. Other investigations showed haemoglobin 14·3 g/dl, white blood cell count $7{\cdot}1 \times 10^9$/l (66% neutrophils, 28% lymphocytes), platelets 306×10^9/l, packed cell volume 0·40 l/l. A course of ultraviolet B irradiation at a suberythema dosage improved his symptoms though some pruritus persisted. Subsequent alkalinization of the bath water with sodium bicarbonate also gave moderate relief. A previous routine blood count in 1987 showed haemoglobin 12·6 g/dl, white blood count $9{\cdot}2 \times 10^9$/l (60% neutrophils, 31% lymphocytes), platelets 98×10^9/l. A bone-marrow examination was performed because of persisting thrombocytopenia over a 3-month period. The bone marrow was hypercellular with normal erythropoiesis and no ring sideroblasts. The myeloid series was increased with a shift to the left but no abnormal infiltration was seen. The megakaryocytes were normal and cytogenetic studies were also normal. Blood results from August 1989 showed a persisting mild anaemia, haemoglobin 12·7 g/dl, white blood cell count 13·7 (62% neutrophils, 29% lymphocytes), platelets 339×10^9/l. He was considered to have a mild form of myelodysplastic syndrome, but a more certain diagnosis would depend on future haematological developments. So far, there have been no conclusive confirmatory changes.

Aquagenic pruritus was first described as a clinical entity in 1979.[1] It consists of itching,[2] or more particularly a prickling, tingling, stinging or burning sensation occurring within 30 min of water contact with the skin and lasting up to 2 h. It usually starts on the lower limbs but may begin anywhere and become generalized. It is not associated with visible skin changes and is only diagnosed after exclusion of physical urticarias such as cold urticaria, symptomatic dermographism, cholinergic urticaria and especially the rare aquagenic urticaria. Psychological changes including aggression, irritability, anger and depression have been noted during attacks of aquagneic pruritus and appear to be a regular secondary feature.[3] Sweating after exercise has been reported as a trigger of aquagenic pruritus. In about one-third of cases, there is a positive family history. The myelodysplastic syndrome[4] refers to a group of conditions involving a low-grade neoplastic proliferation of haemopoietic stem cells which are irreversible and progressive. Clonal alteration of the stem cells produces functionally and structurally defective end cells. The syndrome usually presents with symptoms related to cytopenia but may transform to acute myeloid leukaemia.

The molecular mechanism of aquagenic pruritus has been studied in both isolated aquagenic pruritus and in aquagenic pruritus in association with polycythaemia rubra vera.[3] Local release of acetylcholine and mast cell degranulation in the skin associated with raised blood histamine concentrations have been observed. A low cholinesterase activity in nerve fibres around eccrine sweat glands which increases on exposure to water has been found, suggesting the importance of release of acetylcholine.[5] A working gradually been replaced by white hair. A biopsy of skin from the depigmented area revealed on electron microscopy and incubation with dopa, an absence of melanosomes and melanocytes.

A growth of dark hair within the vitiliginous patch on the forearm was probably due to activation of follicular melanocytes by a non-specific effect of contact dermatitis. Two previous reports[4,5] have described vitiliginous patches developing at sites of contact with DCP, but the possibility of post-inflammatory hypopigmentation was not excluded. In the above case the depigmented patch was confirmed as vitiligo by electron microscopy and dopa reaction. However, it was not clear whether DCP induced the vitiligo by a direct cytotoxic effect on the melanocytes or by unmasking latent vitiligo as a result of the Koebner phenomenon due to contact dermatitis. As the development of vitiligo by DCP is at present unpredictable and the depigmentation may persist indefinitely, it is important to inform all patients of this potential adverse effect before starting treatment.

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hypothesis is that the absorption of an unknown factor through or from the stratum corneum penetrates into the dermis to release acetylcholine from autonomic sympathetic nerve fibres and ultimately histamine and other inflammatory mediators from the mast cells. Increased local fibrinolytic activity[6] has been found in aquagenic pruritus, but it is uncertain whether this is a primary or secondary effect in association with increased acetylcholine or histamine. Platelets may have an aetiological role. In aquagenic pruritus associated with polycythaemia rubra vera, it was proposed that serotonin and prostaglandin E_2 were important as inflammatory mediators.[7] The possible functionally and structurally abnormal platelets in our patient may release inflammatory mediators as part of the response to water contact.

Aquagenic pruritus may be associated with haematological disease and may occur in isolation for some time before a blood disorder becomes apparent.[8] Regular comprehensive checks of the peripheral blood are, therefore, mandatory.

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Persistent vitiligo induced by diphencyprone

Sir, Diphencyprone (DCP) is increasingly being used to treat alopecia areata as encouraging results have been reported and the drug is considered to lack serious adverse effects.[1-3] We describe a patient with alopecia areata who developed persistent vitiligo due to topical therapy with DCP.

A 37-year-old man developed alopecia totalis, loss of eyebrows and eyelashes and widespread thinning of the hair in 1988. The few hairs that remained on the scalp and the limbs had turned white. There was no personal or family history of vitiligo. A full blood count and biochemical profile were normal and the following autoantibody tests were negative: antinuclear factor, rheumatoid factor, smooth muscle antibody, antimitochondrial antibody, thyroid microsomal antibody, thyroglobulin antibody, adrenal antibody and antimelanocyte antibody. Treatment with topical DCP was commenced 2 weeks after applying 2% DCP in acetone to the scalp to achieve sensitization. The scalp failed to react to challenges with 0·1% and 0·2% concentrations of DCP, and in order to differentiate between a failure of sensitization and reduced contact reactivity of the scalp, 0·5% DCP was applied to the flexor aspect of a forearm and on the scalp. A marked reaction with erythema and oedema was evident on the forearm after 3 days but the scalp manifested a slight macular erythema. The forearm reaction subsided after 2 weeks and was replaced 6 weeks later by a depigmented patch of skin with dark hair. The surrounding hair on the forearm remained white. Wood's light examination revealed further patches of depigmentation on the nape of the neck and the midline of the back. A weekly application of 0·5% DCP to the scalp produced the first sign of hair regrowth at 6 weeks and a full head of white hair together with regrowth of eyebrows and eyelashes at 4 months. There has been no relapse of alopecia for 2 years after discontinuing DCP therapy and the depigmented areas remain unchanged. However, dark hair in the depigmented patch on the forearm has

British Journal of Dermatology (1990) **123**, 417–418.

Book Reviews

Andrew's Diseases of the Skin. Clinical Dermatology. Eighth Edition. H.L.ARNOLD JR, R.B.ODOM AND W.D.JAMES (1990). Philadelphia: W.B.Saunders Co. Pp. 1062. Illustrations, 1196. ISBN 0-7216-2424-3. Price £78.50.

Thirty years ago for a young doctor *Andrew's Diseases of the Skin* was one of the few easy to read comprehensive guides to Dermatology. The latest eighth edition continues to follow this pattern and there is said to be a unified coherent approach to the book as well as to the philosophy of ones' approach to skin disease in general.

There is a strong clinical orientation for 'desk top' rather than library use and it is intended to be useful to students, interns, residents, medical specialists, family practitioners and all physicians including dermatologists. Since the last edition in 1981 there are many major changes, particularly with regard to aetiopathogenesis and treatment.

About half the text has been rewritten to cover particularly advances in molecular biology, immunology and understanding of the skin as an immunologically active organ. There are also advances in dermatopathology and immunohistochemical analysis incorporated into the new edition.

New therapeutic advances such as retinoids, antiviral agents, laser technology, newer antibiotics, minoxidil, interferons and photochemotherapy are all mentioned in the text. The book contains many new diseases such as AIDS and other retroviral infections with over a hundred newly described disorders. There are new chapters on X-ray therapy and dermatological surgery.

The references are up to date, some even from 1988 and 1989, many are from the *Archives of Dermatology*, *British Journal of Dermatology* and the *Journal of the American Academy*. They are interspaced throughout the book rather than at the end of each chapter as was the case in previous editions.

The authors hope that the book is still the eminently practical 'what is it' and 'how to' book that it has always been and advise the reader to 'enjoy'.

The new Andrews is certainly a pleasure to handle and browse through and is very good value for money. Each page is printed in two columns, making it easy to read. The text is interspersed with many excellent black and white photographs.

N.MORLEY

Flaps for the Central Face. S.J.SALASCHE AND W.J.GRABSKI (1990). New York: Churchill Livingstone. Pp. 93. ISBN 0-443-08661-3. Price £17·50.

This is a very useful, small, illustrated paperback describing flap repairs around the lips and on the nose. It has been written by two practising American dermatological surgeons for dermatologists interested in skin surgery. There is a good introductory chapter on the principles of flap repair, anatomy, local anaesthetic techniques and cosmetic consideration. Two other chapters deal with flap repairs on the nose and lips and each of the three chapters ends with a useful list for further reading. Within each chapter three or four different types of closure are illustrated for each part of the lips or nose using good line drawings to show the defect, the incision lines, the areas of undermining and the final closure design. For example on the upper lip there is a description of a rotation flap, A to T closure, cosmetic unit advancement flap, cheek advancement flap and two types of transposition flap. At the start of each is a brief outline of the reasons for choosing this particular closure. The stages of procedures are also described in a step wise fashion in clear and concise English.

My criticisms of this book are few. It succeeds in doing what it set out to do, i.e. concisely describe the type of flap designs available for the repair of tumour excision defects on the nose and lips. Because there are no photographs the artist has been able to demonstrate principles and ignore potential hazards such as the undesirable movement of the philtrum and lip centre, or so called Cupids bow, during upper lip surgery. If I had to find faults it would be the lack of an adequate description of the potential complications that can occur with each procedure and how to avoid them.

I strongly recommend this book, it is light enough to be kept in a briefcase as a ready reference and it deals with two sites on the face where skin tumours are common and closures are difficult because of the need to avoid distortions of the important cosmetic free margins of the nose and lips. Any dermatologist excising tumours on the face and wishing to be more adept in using flaps to repair the defects created would be wise to buy this book.

C. Lawrence

A History of St John's Hospital for Diseases of the Skin—1963–1988. P.D. Samman (1990). Oxford: Radcliffe Medical Press Ltd.

This charming and timely vignette is an account of the last years of working at St John's Hospital. Clearly it is an attractive publication at a time when one door has closed and another door has opened in British dermatology in London. Dr Samman has invited a number of individuals to write brief accounts of working in St John's and the result is a very pleasant mixture of styles and memories. If you wish to see the programme for the Diploma Course in the summer term 1985, to know Malcolm Greaves' home address, or to see staff lists and selected publication lists you will find this here.

The book is a hardback and a pleasantly produced publication. Many individuals with a sentimental attachment to the Lisle Street building may wish to purchase it.

Rona M. Mackie

Management of Blistering Diseases. Edited by F. Wojnarowska and R.A. Briggaman (1990). London: Chapman and Hall. Pp. 308. ISBN No. 0-412-28990-3. Price £65.00.

This book must become the standard reference on bullous diseases. Over the last 20 years there have been major advances in the diagnosis and consequently management of the blistering disorders and in this publication there are chapters written by those who have made contributions from Britain, the United States and Europe.

The first chapter is a very erudite and interesting historical review by Professor Karl Holubar of the terminology applied to blistering disorders which have fascinated medical people from the days of Hippocrates.

Part one deals with the immunological bullous diseases where immunofluorescence techniques have greatly improved diagnostic accuracy in this group of diseases and now with monoclonal antibodies, the specific antigenic components of the junction are being explored and this should lead to an understanding of the aetiological factors involved and also the function of the skin. There are detailed descriptions of the techniques which are applicable to skin pathology. These are then followed by a section on the diagnosis and treatment of the major immunological bullous diseases in which there is a little overlap, but these sections are by a number of different authors and the contributions are all well written, which says much for the skills of the editors.

Part two deals with the genetically determined bullous diseases and again chronicles the great advances that have been made with immunoelectron microscopy in the diagnosis and classification of these often distressing disorders. In managing those young patients, and in particular their families, accurate diagnosis is of the utmost importance and this is well covered including the place of prenatal diagnosis and the possibilities of genetic counselling. There is also a very sympathetic caring section on management of children with epidermolysis bullosa with much useful practical advice. For the future there are reports on the advancing areas of research which are revealing molecular abnormalities in certain types of epidermolysis bullosa, which may one day be capable of modification at the gene level.

The final section deals with a mixed group of disorders which range from erythema multiforme, lupus erythematosus, porphyrias, bullous erythema multiforme to bullous eruptions occurring in association with chronic renal disease and drugs. As in all the other sections there is useful advice about the treatment and management of some of these potentially lethal conditions. The book is well illustrated in black and white and each section has a good list of references.

I enjoyed reading this book which should be in all departmental libraries for reference and I think many practising dermatologists would find a personal copy very interesting, particularly if they have lived through the revolution which has taken place in the last 20 years on diagnosis and classification of blistering disorders. It will also be of great value to all who are involved in the management of patients with blisters.

G.W. Beveridge

British Journal of Dermatology (1990) **123**, 419.

News and Notices

The New York Academy of Sciences
Molecular and Structural Biology of Hair Growth
23–25 January 1991, Arlington, VA, U.S.A.

A conference sponsored by the New York Academy of Sciences on Molecular and Structural Biology of Hair Growth will be held at the Crystal Gateway Marriott in Arlington, VA, 23–25 January 1991. In addition to formal lectures and open discussions there will be a contributed poster session and the deadline for submission of poster abstracts is 15 September 1990. The entire abstract including title, authors and affiliations must be typed single-spaced and contained within a rectangle that measures 5 × $4\frac{3}{8}$ inches. The abstracts should be sent to Dr K. S. Stenn, Yale University, 333 Cedar Street, New Haven, CT 06510-8058, U.S.A. For further information contact: Marketing Department, the New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, U.S.A.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
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microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
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British Journal of Dermatology, Volume 123, Number 3, September 1990

CONTENTS

PAGE

Effect of topical retinoic acid on the interleukin 1α and β immunoreactive pool in normal human epidermis … 283
D.CHATELLARD GRUAZ, L.DIDIERJEAN, D.GUMOWSKI-SUNEK AND J-H.SAURAT

DNA content and K_s8.12 binding of the psoriatic lesion during treatment with the vitamin D_3 analogue MC903 and betamethasone … 291
S.DE MARE, E.G.J.M.DE JONG AND P.C.M.VAN DE KERKHOF

Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides … 297
FRANCES LAWLOR, N.P.SMITH, R.D.R.CAMP, K.B.BACON, ANNE KOBZA BLACK, M.W.GREAVES AND A.J.H.GEARING

Elastic fibre damage induced by low-dose D-penicillamine … 305
K.L.DALZIEL, S.M.BURGE, P.A.FRITH, T.J.RYAN AND A.MOWAT

Invasive migration of epidemic Kaposi's sarcoma cells *in vitro* … 313
C.G.SCHIRREN, W.K.ROTH, R.HEIN, S.WERNER, T.KRIEG AND O.BRAUN-FALCO

Neutrophil zinc levels in psoriasis and seborrhoeic dermatitis … 319
R.S.C.LEUNG, A.J.TURNBULL, J.A.TAYLOR, R.RUSSELL-JONES AND R.P.H.THOMPSON

Autologous mixed lymphocyte reaction is reduced in patients with psoriasis … 325
T.TERUI, M.ROKUGO, S.AIBA, T.KATO AND H.TAGAMI

Differential enhancement of interferon-γ-induced MHC class II expression of HEp-2 cells by 1,25-dihydroxyvitamin D_3 … 333
K.TAMAKI AND K.NAKAMURA

Pentoxifylline inhibits the proliferation of human fibroblasts derived from keloid, scleroderma and morphoea skin and their production of collagen, glycosaminoglycans and fibronectin … 339
B.BERMAN AND M.R.DUNCAN

Analysis of side-effects of medium-and low-dose cyclosporin maintenance therapy in psoriasis … 347
M.A.DE RIE, M.M.H.M.MEINARDI AND J.D.BOS

White piedra: evidence for a synergistic infection … 355
K.M.ELLNER, MOLLIE E.MCBRIDE, D.C.KALTER, J.A.TSCHEN AND J.E.WOLF, JR

Ultrasound velocity in human fingernail and effects of hydration: validation of *in vivo* nail thickness measurement techniques … 365
A.Y.FINLAY, B.WESTERN AND C.EDWARDS

The prophylactic use of cyclooxygenase inhibitors in recurrent herpes simplex infections … 375
M.WACHSMAN, L.AURELIAN AND J.W.BURNETT

Effects of haem arginate on variegate porphyria … 381
K.TIMONEN, P.MUSTAJOKI, R.TENHUNEN AND J.LAUHARANTA

Successful treatment of eruptive pyoderma gangrenosum with intravenous vancomycin and mezlocillin … 389
S.KANG AND J.S.DOVER

Skin manifestations in congenital deficiency of leucocyte-adherence glycoproteins (CDLG) … 395
P.C.M.VAN DE KERKHOF AND C.M.R.WEEMAES

An unusual case of acquired localized argyria … 403
L.SHALL, A.STEVENS AND L.G.MILLARD

Granulomatous cheilitis and Crohn's disease … 409
Y.KANO, T.SHIOHARA, A.YAGITA AND M.NAGASHIMA

Correspondence

Kawasaki disease in a young adult with a perineal rash … 413
L.MACHET, L.VAILLANT, H.GOUTAL, G.LANTERNIER, R.KHALLOUF, J.L.CHAGNON AND G.LORRETTE

Aquagenic pruritus and the myelodysplastic syndrome … 414
J.A.MCGRATH AND M.W.GREAVES

Persistent vitiligo induced by diphencyprone … 415
P.DUHRA AND I.S.FOULDS

Book Reviews … 417

News and Notices … 419

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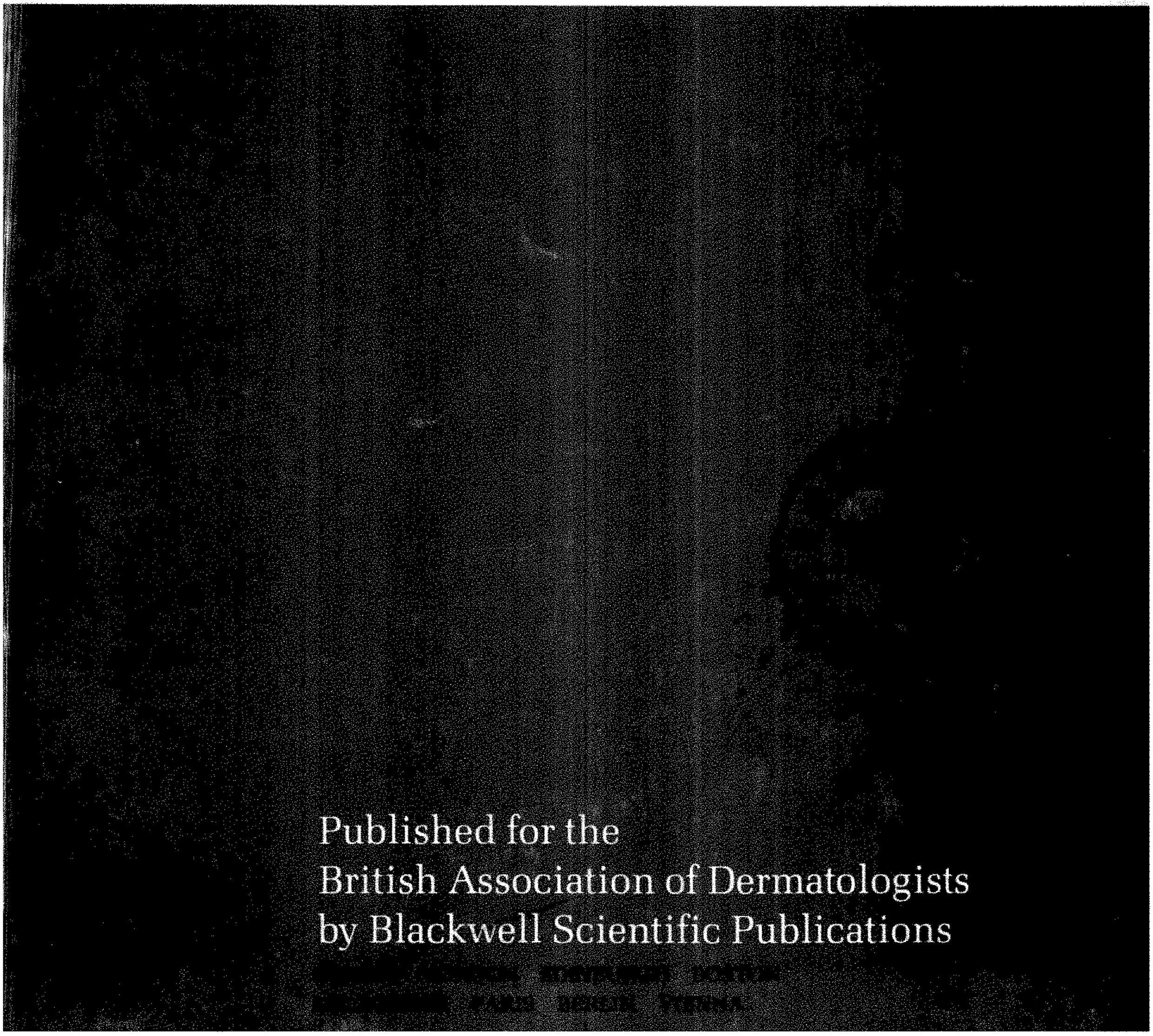

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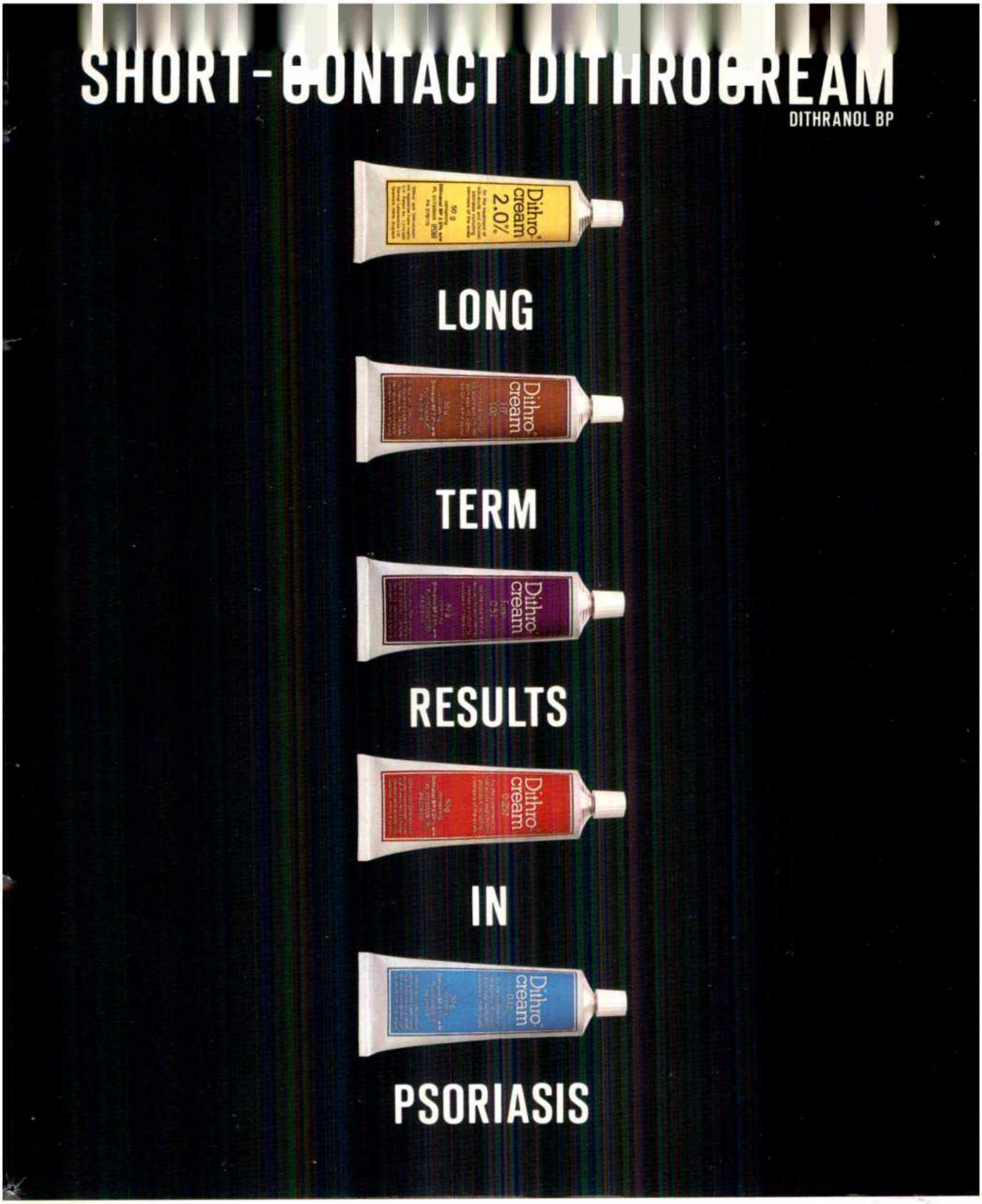

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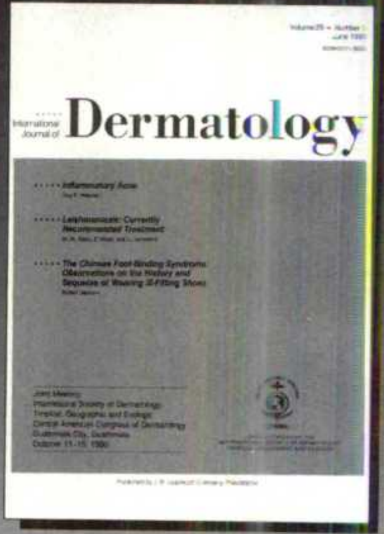

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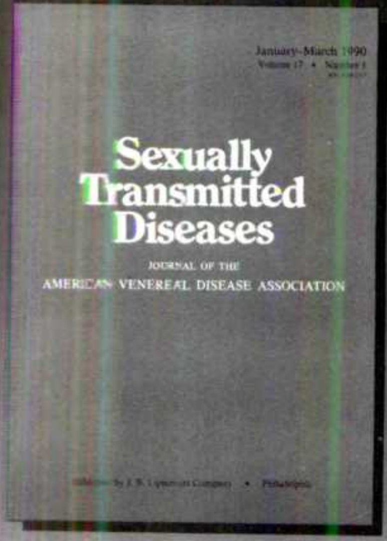

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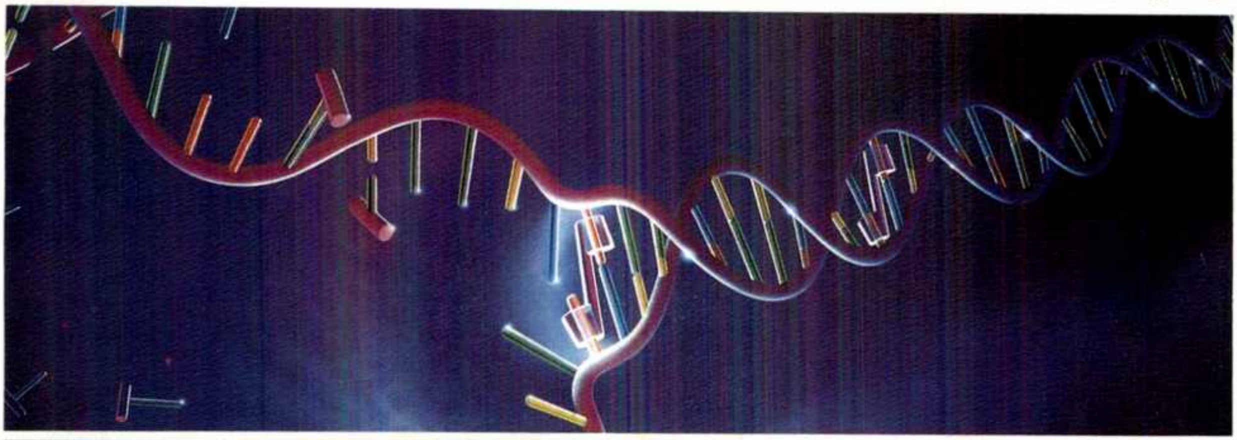

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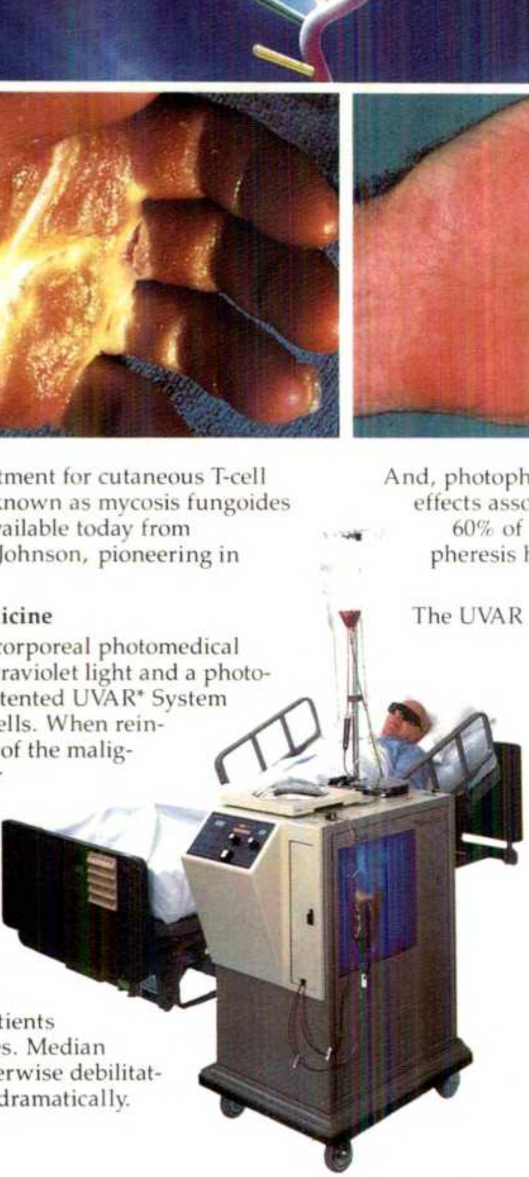
1. Edelson, R.L.: Light-activated Drugs. Scientific American 259(2):68-75, August 1988.



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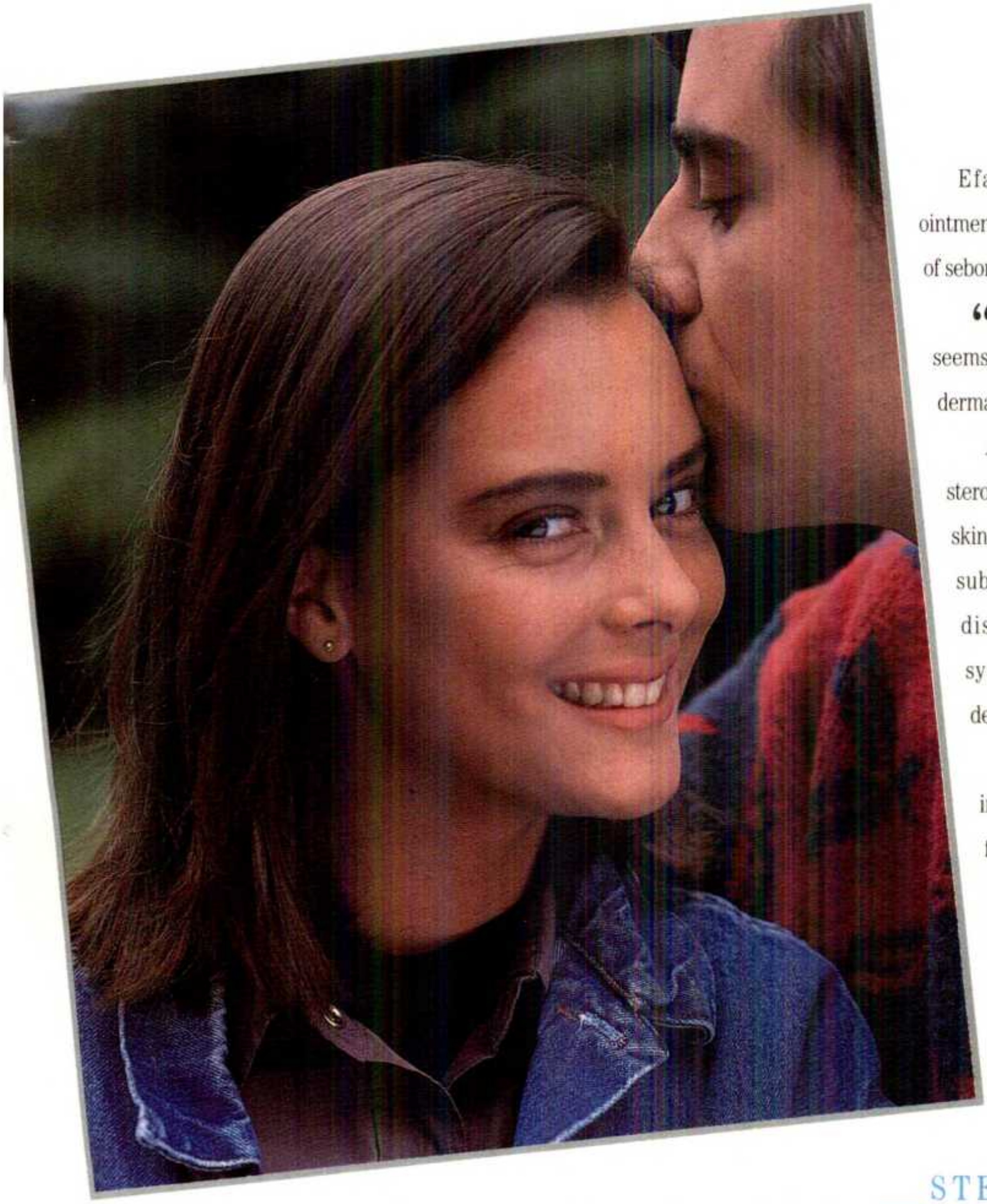

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REFERENCES

1. Boyle J, Burton JL, Faergemann J. Br Med J 1986; 292:28
2. Data on File. Scotia Pharmaceuticals Ltd.

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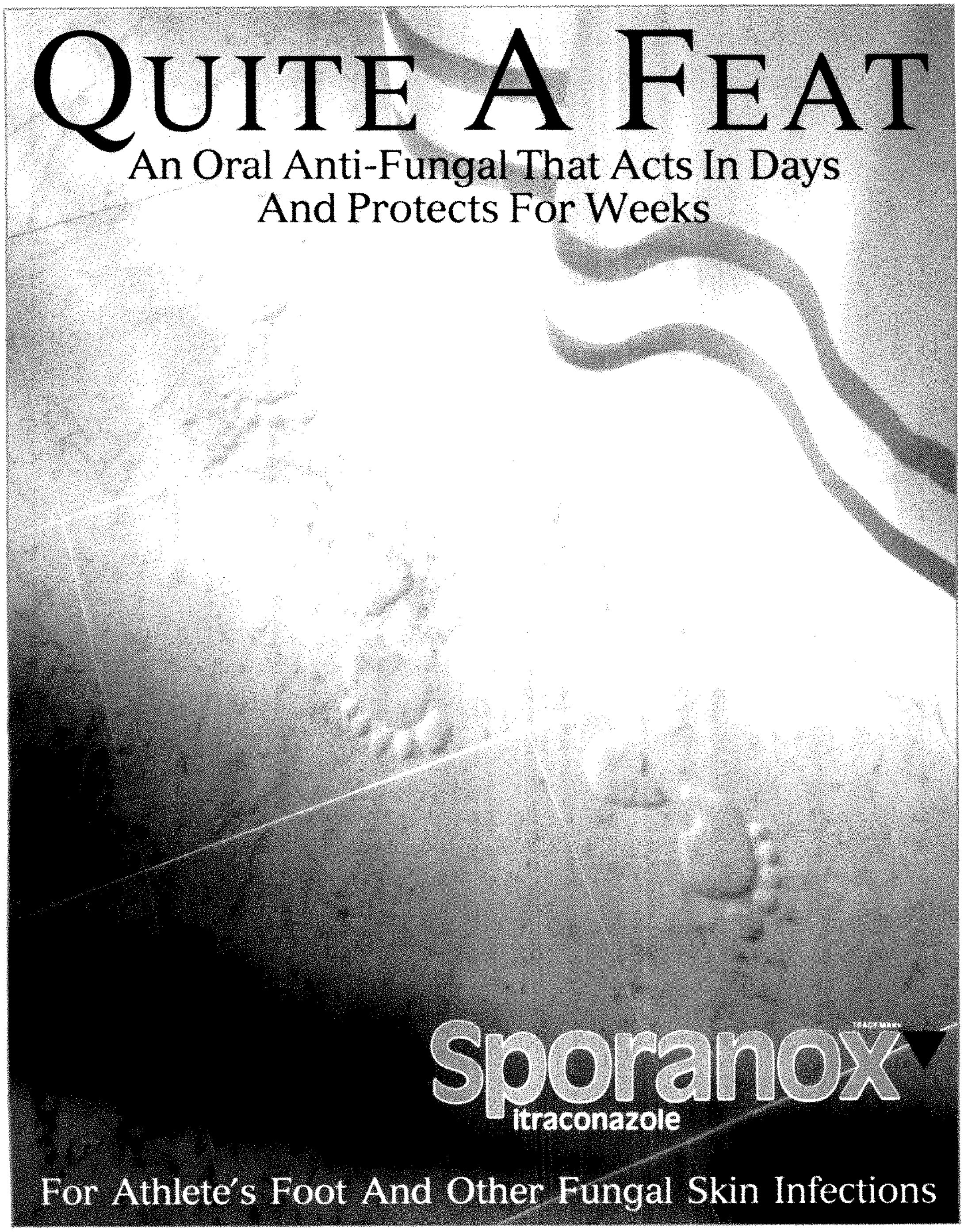
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Editors: Nancy B. Esterly, M.D.; *Childrens Hospital of Wisconsin*
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REFERENCES

1. Stewart JCM *et al.* Data on file – Scotia Pharmaceuticals Ltd. 2. Morse PF *et al.* Br J Dermatol 1989; 121: 75-91. 3. Wright S, Burton JL. Lancet 1982; ii: 1120-1122. 4. Schalin-Karrila M et al. Br J Dermatol 1987; 117: 11-19. 5. Biagi PL *et al.* Drugs Exptl Clin Res 1988; **XIV**(4): 285-290. 6. Bordoni A *et al.* Drugs Exptl Clin Res 1988; **XIV**(4): 291-297.

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British Journal of Dermatology (1990) **123**, 421–430.

Autoantibodies to nucleolar antigens in systemic scleroderma: clinical correlations

MARIA BŁASZCZYK, MARIA JARZĄBEK-CHORZELSKA, STEFANIA JABŁOŃSKA, T.CHORZELSKI, ZOFIA KOŁACIŃSKA-STRASZ, E.H.BEUTNER* AND V.KUMAR*

Department of Dermatology, Warsaw School of Medicine, Poland and * Department of Microbiology, State University of New York at Buffalo, U.S.A.

Accepted for publication 23 April 1990

SUMMARY

Indirect immunofluorescence (IIF) and double immunodiffusion (DID) were performed on the sera of 64 patients who had a nucleolar immunofluorescence pattern on HEp-2 cells. Forty-nine of the sera were from 296 patients with systemic scleroderma (SSc) and 15 sera were from 214 patients with systemic lupus erythematosus (SLE). A homogeneous nucleolar staining pattern was found in 45 of the 64 sera (70·3%), a clumpy fluorescence associated with fibrillarin antibody in 14 (21·8%) and a speckled pattern was found in five of the sera (7·8%).

There was a clear correlation between the sera which showed a homogeneous nucleolar staining pattern with symptoms of the polymyositis/scleroderma overlap syndrome that differed from SSc with concomitant myositis. The clumpy pattern was mainly associated with diffuse scleroderma and the speckled pattern with limited scleroderma (previously called acrosclerosis).

Antinucleolar antibodies are characteristic markers of systemic scleroderma (SSc).[1–5] Recently, various types of nucleolar antigens have been identified as targets for these antibodies. The four main nucleolar antigens are PM-Scl (proteins of 20–110 kDa that are not fully characterized and are related to preribosomes), fibrillarin a protein of 34 kDa (U3 RNP), RNA polymerase 1 and RNase P.[6,7] The antibodies to these antigens have to be detected either by immunoprecipitation or double immunodiffusion (DID). They can also be recognized by their characteristic pattern using indirect immunofluorescence (IIF) on HEp-2 cells. Antibodies to PM-Scl produce a homogeneous nucleolar and weak nucleoplasm staining. The chromatin in the metaphase of the cells is not stained. Antibodies to fibrillarin produce bright clumpy nucleolar staining but not that of the nucleoplasm and the metaphase cells show strong fluorescence of the condensed chromatin. Antibodies to RNA polymerase 1 produce speckled or punctate nucleolar and weak

---

Correspondence: Dr Maria Błaszczyk, Deparment of Dermatology, Warsaw School of Medicine, Koszykowa 82a, 02-008 Warsaw, Poland.

nucleoplasm staining, with several dots in the area of condensed chromosomes of the dividing cells corresponding to the so-called nucleolar organizing region.[8,9] Antibodies to the newly recognized RNase P,[7] formerly known as 7-2 RNP,[10,11] produce a bright nucleolar fluorescence that is homogeneous.

So far there are only a few reports on the correlations between the types of nucleolar autoantibodies and the clinical features of SSc.[6,9,12–16] The correlations of the three main types of nucleolar antibodies in a series of 46 cases of SSc were described by Reimer *et al.*,[9] who found clumpy antinucleolar antibodies in 22, a homogeneous pattern in 11 and a speckled type in 13.

We report considerable differences in the prevalence of the three types of nucleolar antibodies and their clinical correlations in systemic scleroderma and point to the association of a homogeneous nucleolar pattern with the overlap cases.

METHODS

Patients

The sera from 296 cases of systemic scleroderma (SSc), 40 cases of mixed connective tissue disease (MCTD, Sharp syndrome) and 214 patients with systemic lupus erythematosus (SLE) were studied. The cases of SSc were classified as either diffuse or limited according to the ARA criteria.[17,18] MCTD was classified according to the criteria of Sharp *et al.*[19,20] and SLE according to the criteria of the ARA.[21] The patients were classified as having diffuse scleroderma if the skin was involved on the face, extremities and the trunk, often with symmetrical hyper- and hypopigmentation. The course of the disease was usually more rapid and severe in this type.[17,22]

Indirect immunofluorescence (IIF)

Sera were screened for antinuclear antibodies (ANA) using monkey oesophagus at a starting dilution of 1:10. The staining patterns were evaluated using HEp-2 cells (Immco Diagnostic, Buffalo, NY, U.S.A.) as previously described.[5,9] The conjugate used was a goat antihuman IgG (Heintel, Vienna, Austria) with a fluorescein to protein ratio of 2·2.

Double immunodiffusion (DID)

Calf thymus extract was used for the detection of Scl-70 antigen as has been previously described.[23] For other antigens, including PM-Scl, calf thymus was homogenized with two volumes of cold phosphate-buffered saline at pH 7·2 at 4°C. The homogenate was stirred overnight at 4°C and centrifuged at 12,000 rpm for 60 min, and following centrifugation the supernatant was precipitated in ammonium sulphate. The precipitate was then dissolved in 0·01% Tris buffer at pH 7·2 and dialysed overnight with 2–3 changes of the buffer and subsequently lyophilized. For DID, 150 mg/ml of calf thymus extract was used and the assays were performed as has been described.[24]

RESULTS

The results of the immunofluorescence studies on the frequency of the three nucleolar fluorescence patterns in the 64 cases where they were positive are shown in Table 1. The homogeneous staining pattern (Fig. 1) was found in the majority of sera (70·3%). A clumpy fluorescence pattern (Fig. 2) was found in 21·8% of the sera and a speckled pattern (Fig. 3) was less frequently found in 7·8% of the sera.

TABLE 1. Frequency of three nucleolar fluorescence patterns in 64 cases of collagenoses positive for antinucleolar antibodies

IIF nucleolar pattern	Total (%)	Limited scleroderma	Diffuse scleroderma	Overlap and scleromyositis cases	SLE	SLE suspected not confirmed
Homogeneous	45(70·3)	6	0	30*	2	7
Clumpy	14(21·8)	0	6	4	2	2
Speckled	5(7·8)	3	0	0	2	0

* In one case there was coexistence of ACA, in two cases clumpy nucleolar pattern, and in three cases La antibodies.

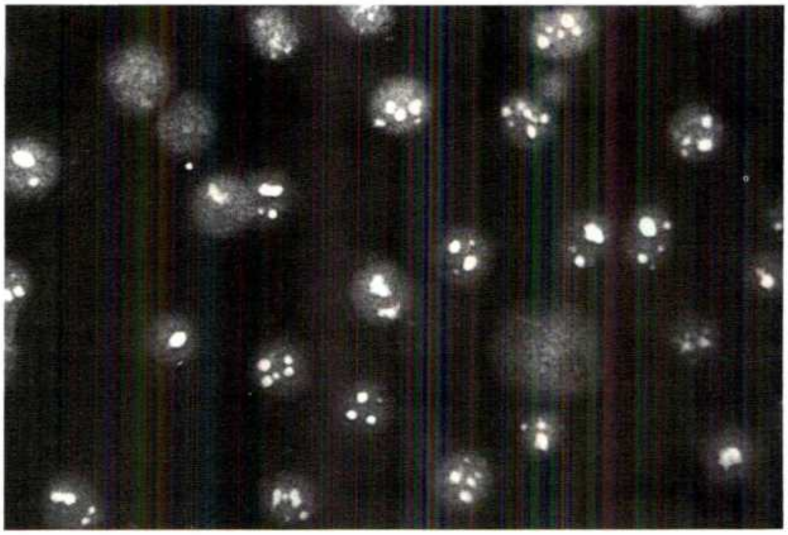

FIGURE 1. Strong homogeneous staining of nucleoli, and weakly stained nucleoplasm. Metaphase cell negative for chromosomal staining, but showing diffuse fluorescence of nucleoplasm.

The homogeneous nucleolar pattern was found in conjunction with other antibodies in six patients. The presence of a high titre of ACA of 5120 was detected in one case by IIF and confirmed by immunoblot with the use of recombinant kinetochore antigen.[25] In this patient (Figs 4a and b) the vascular involvement was predominant.[26] In three cases, La antibody was confirmed by DID. These patients had no signs of the sicca syndrome and did not differ from the other patients with the PM-Scl overlap syndrome. In two cases of PM-Scl overlap syndrome, coexistence of fibrillarin antibody could be presumed since some mitoses were stained in a characteristic manner in a clumpy pattern, while PM-Scl antibody was confirmed by DID.

There was a correlation between the homogeneous nucleolar staining pattern and unclassified cases with sclerodermoid features, i.e. polymyositis-scleroderma overlap or scleromyositis (30 of 45 cases, 66·6%). However, six cases with this pattern were of limited scleroderma type, two were SLE and seven others were suspected of SLE but this diagnosis could not be confirmed.

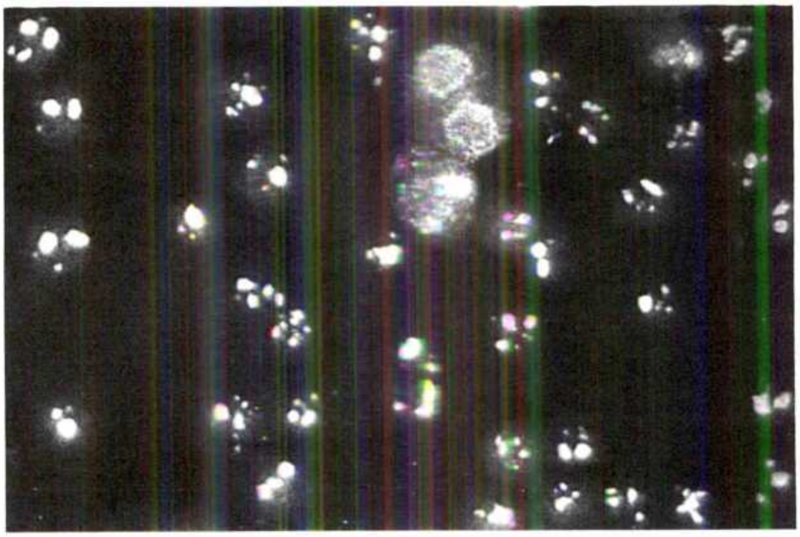

FIGURE 2. Clumpy nucleolar pattern. Very strong, somewhat irregular staining of nucleoli, frequently with nucleolar dots, and usually negative nucleoplasm. In metaphase cells, strong staining of condensed chromosomes.

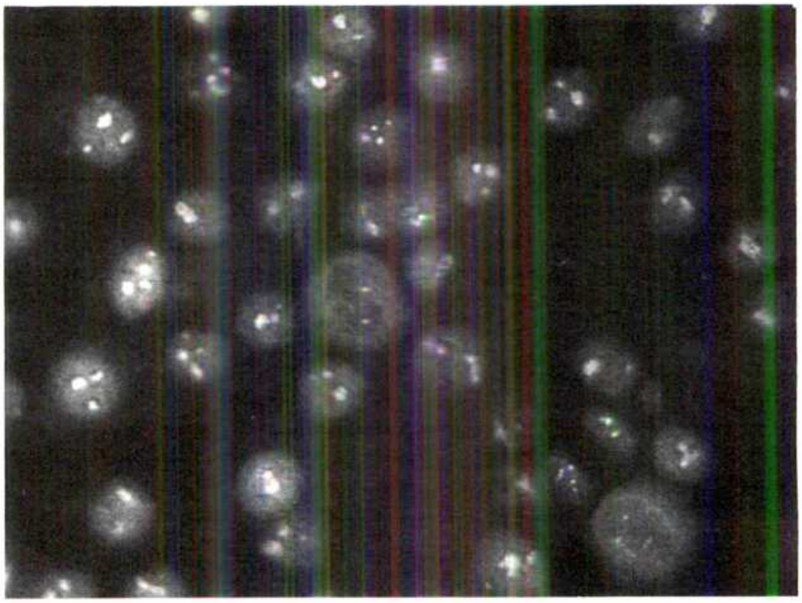

FIGURE 3. Speckled (punctate) nucleolar staining. Nucleoli filled with speckles, nucleoplasm frequently weakly stained. In metaphase cells, several fluorescent dots in the area of condensed chromosomes (nucleolar organizing region).

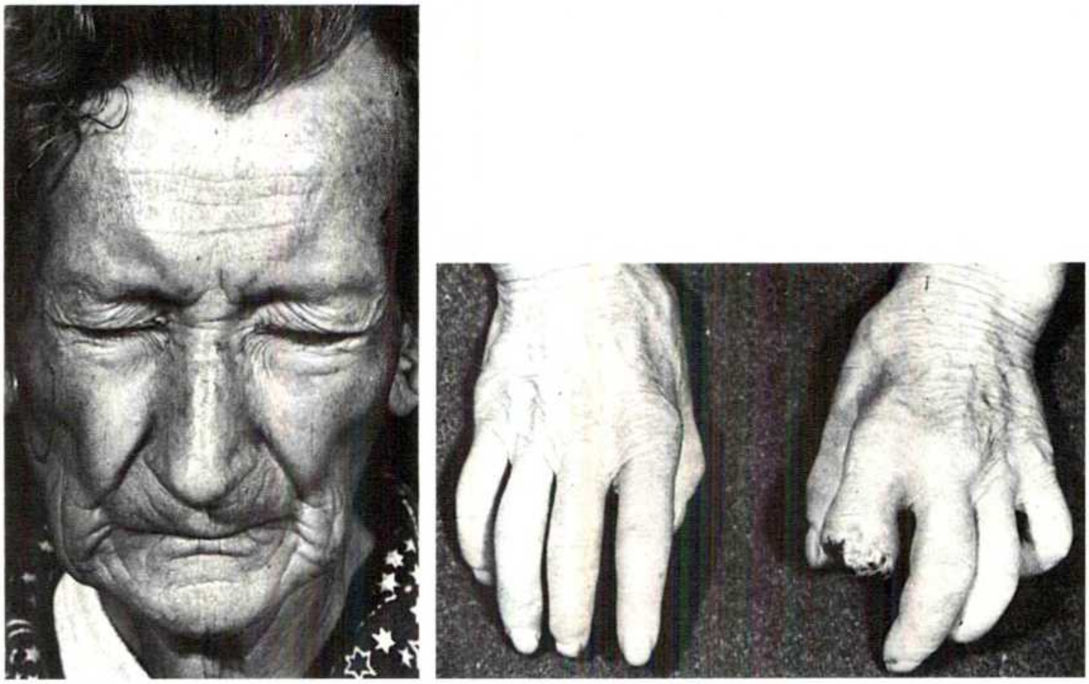

FIGURE 4. A 75-year-old female positive for both PM-Scl antibody and ACA. (a) The face with no typical scleroderma features; (b) the hands with extensive osteolysis, sclerodactyly, and pronounced muscle atrophy on the dorsa of the hands, without cutaneous induration.

The clumpy pattern was associated with diffuse scleroderma in six patients, four females and two males. One of these was a 13-year-old boy. In four of the patients the clinical features were similar to those of Sharp's syndrome with more pronounced sclerodermoid symptoms. Two cases were of SLE and one patient had multiple autoimmune abnormalities. One was the healthy sister of a patient with SLE.

The speckled pattern was associated either with limited scleroderma or with SLE. In none of the 40 cases of Sharp's syndrome were antinucleolar antibodies found.

TABLE 2. Clinical correlations between cases with nucleolar homogeneous fluorescence positive and negative for PM-Scl antibodies using double immunodiffusion (DID)

DID (no. of cases)	Limited scleroderma	Overlap and scleromyositis cases	SLE	SLE suspected not confirmed
PM-Scl positive (39)	4	30*	2	3
PM-Scl negative (6)	2	0	0	4

* Coexistence of La antibodies in three cases.

The sera producing clumpy and speckled staining on HEp-2 cells were invariably negative on DID. Of the 45 sera producing the homogeneous pattern, 39 were positive using DID for PM-Scl antibody (Table 2). Although there was a correlation between the presence of PM-Scl antibodies and scleromyositis, patients whose sera produced homogeneous staining on HEp-2 cells but were negative using DID did not show overlap features. The clinical characteristics of patients with scleromyositis who were positive for PM-Scl, as compared with SSc cases positive for Scl-70 or for ACA, are shown in Table 3.

The difference between the three groups was the presence of myositis in all PM-Scl overlap cases. The cutaneous lesions were mostly oedematous or atrophic, with some sclerodermoid features, especially on the face and hands (Figs 5a and b). Telangiectasia was present in 23% of the cases, and was less frequent than in those cases positive for ACA. However, calcinosis was

TABLE 3. Clinical characteristics of patients with scleromyositis who were positive for PM-Scl antbodies, vs. those with systemic scleroderma positive for Scl-70 or anticentromere antibodies

Clinical and laboratory findings	PM-Scl overlap cases/scleromyositis $n = 27$	Systemic scleroderma Scl 70 positive $n = 36$	 ACA positive $n = 33$
Age at onset (years) (mean)	6–60 (26·6)	15–56 (35·3)	12–66 (35·3)
Duration of disease (years) (mean)	2–29 (9·6)	1–33 (9·5)	1–38 (13·2)
Raynaud's phenomenon as a first symptom	14/25	29/36*	33/33***
Cutaneous lesions			
Typical limited scleroderma	0/27	12/36***	31/33***
Typical diffuse scleroderma	0/27	24/36***	0/33
Sclerodermatoid features†	24/27	0/36***	2/33***
Signs of dermatomyositis	10/23	0/36***	0/33***
Telangiectasia	6/26	21/36**	27/33***
Calcinosis	8/26	14/36	14/33
Arthritis or arthralgia	27/27	27/36**	27/33*
Muscle involvement	27/27	12/36***	4/33***
Visceral involvement			
Oesophagus	3/27	24/36***	16/33***
Heart	3/27	22/36***	21/33***
Lung	4/27	24/36***	12/33*
Kidney	0/27	4/36	1/33
Capillaroscopy			
R loops only	5/21	4/33	18/31**
R + S loops	0/21	29/33***	11/31***
R + DM loops	11/21	0/33***	1/31***

† Sclerodermatoid features: either puffy, somewhat indurated fingers, or no hand indurations, sometimes with muscle atrophy, and often furrowing around the lips, thinning of the nose and/or slightly mask-like facies.

Statistically significant difference from scleromyositis (function test): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

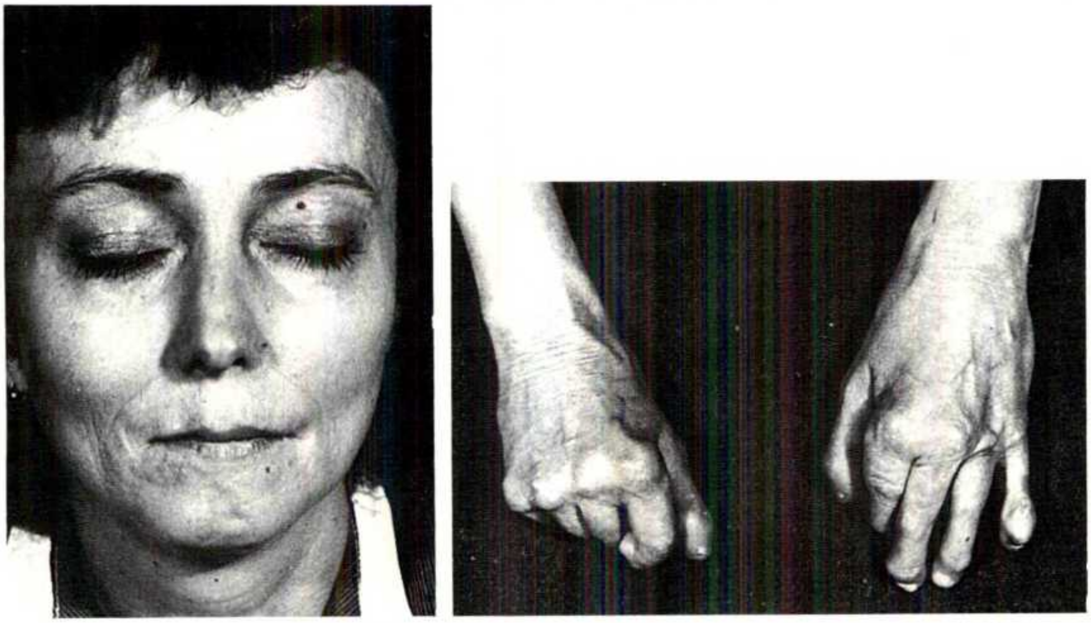

FIGURE 5. A 41-year-old female, whose disease started at the age of 12, initially recognized as dermatomyositis, and ran a chronic, relatively benign course. PM-Scl antibody was the marker disclosed by DID and IIF on HEp-2 cells. (a) The face with slight atrophic changes and minimal sclerodermoid features; (b) the hands showing deformities and muscle atrophy, with digital contractures, but without any induration and osteolysis.

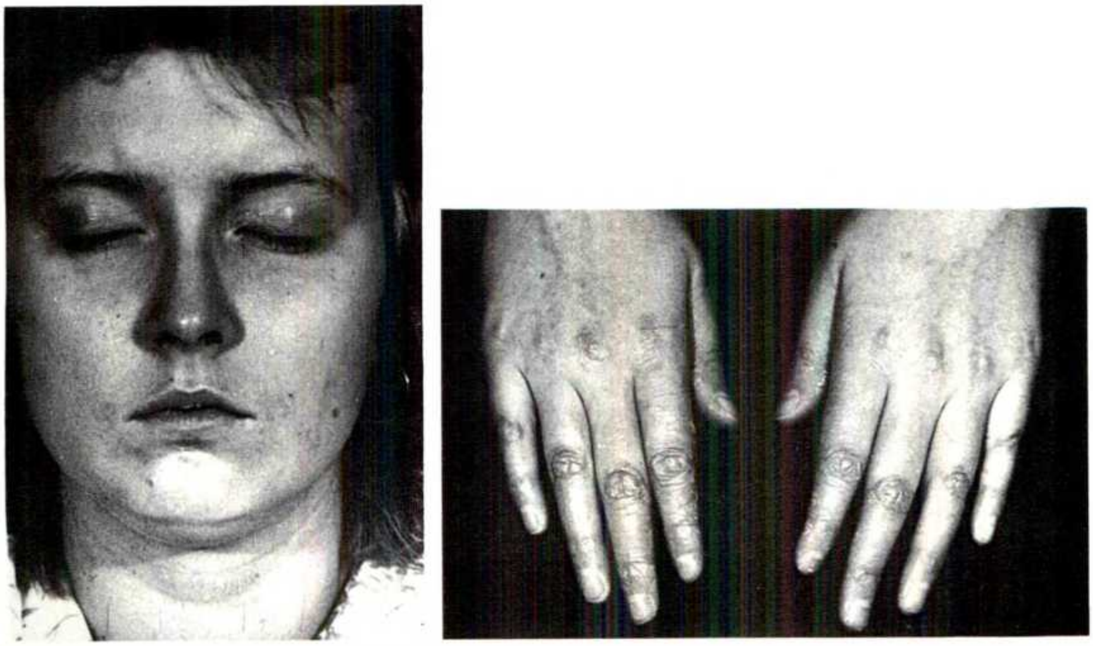

FIGURE 6. A 19-year-old girl, in whom the disease started at the age of 9 and was recognized as systemic scleroderma with pronounced muscle involvement. The immunological marker was PM-Scl antibody. (a) The face with no sclerodermoid features; (b) the hands with slight atrophy of distal phalanges, but with no osteolysis. Pronounced Gottron sign.

detected with a similar frequency. In 10 of 23 patients there were some cutaneous signs of dermatomyositis but with no muscle pain or weakness at the time of study (Figs 6a and b). However, every patient had variable symptoms of myositis at some time or other in the course of the disease. Capillary microscopy in 52·3% of the cases showed giant capillaries and telangiectasia.

DISCUSSION

In our series of 296 patients with systemic scleroderma (SSc), a nucleolar pattern was found on immunofluorescence staining of HEp-2 cells in 49 (16·5%) of the cases. This percentage is somewhat higher than the 8% found by Reimer *et al.*[9] though some authors have found a prevalence of nucleolar antibodies as high as 45%[5] and 73%.[27] This discrepancy could be explained by having included the staining of nucleoli produced by Scl-70 antibody, in addition to the characteristic fluorescence of the nucleoplasm.[23] In our studies we found a high prevalence of the homogeneous nucleolar pattern in 70% of our patients. This type of staining was the least frequent in the study of Reimer *et al.*,[9] who reported the highest frequency to be that of a clumpy pattern, being 47·8% as compared to that of 21·8% in our series. The speckled pattern was found by us to be 7·8% of the sera examined and by Reimer *et al.* in 28·2%.[9] This discrepancy is due to differences in the SSc subsets studied.

The clumpy pattern of nucleolar fluorescence was associated in some of the cases with severe diffuse scleroderma, but also with other collagen disorders. The speckled pattern was found by Reimer *et al.*[9] to be associated with diffuse scleroderma of short duration and a high prevalence of internal organ involvement. In our series, the pattern was seen in only three cases of limited SSc and in two of the cases of SLE. This nucleolar antibody was also found by others in the sera of SLE cases.[28]

We confirmed by DID that not all sera producing a homogeneous nucleolar staining are positive for PM-Scl antibody. We found some clinical differences between the patients with the homogeneous nucleolar pattern who were either positive or negative for PM-Scl antibody using DID. The majority of the patients whose sera were positive for PM-Scl, 30 of 39 cases, had the clinical features of scleromyositis or PM-Scl overlap syndrome. This agrees with the study of Meurer *et al.*[16] who found PM-Scl antibody in six of eight cases with this diagnosis. PM-Scl antibody may appear in conjunction with other antibodies. In our series, the presence of ACA was associated with predominant vascular involvement, as previously reported.[26] The coexistence of fibrillarin antibody was found in two cases of PM-Scl overlap syndrome that were similar to other cases with only the PM-Scl marker.

Our cases of scleromyositis differed in their clinical characteristics from those with typical SSc. In all patients, there was muscle involvement of variable severity, often present only for some periods in the disease. The cutaneous indurations were slight or absent, but the patients had some sclerodermoid features, particularly on the face and/or the hands. Telangiectasia was much less frequent than in SSc and in a proportion of patients, there were cutaneous symptoms of dermatomyositis with periorbital erythema and Gottron sign. The onset of the disease also differed from that in SSc by an absence of Raynaud's phenomenon in more than 40% of the patients, although this developed later on in the course of the disease. Calcinosis was present in about 30% of the patients, but this finding is consistent both with scleroderma and dermatomyositis. Capillaroscopy showed that in 50% of the patients, in addition to Raynaud's loops, there were giant distorted capillaries with haemorrhages. Visceral involvement in this group was significantly less frequent than in SSc, but arthralgia or arthritis was present in all cases. An important difference from SSc with pronounced myositis positive for Scl 70 was a relatively benign course. Another was the frequency in childhood.

In conclusion, our study has shown the importance of distinguishing the three different antinucleolar antibodies using indirect immunofluorescence and double immunodiffusion. The PM-Scl antibody which produces a homogeneous nucleolar pattern on HEp-2 cells and is positive on DID is a marker of polymyositis-scleroderma overlap syndrome, which usually runs a relatively benign course and occurs more frequently in children.

ACKNOWLEDGMENT

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Endemic pemphigus foliaceus in western Parana, Brazil (1976–1988)

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SUMMARY

Endemic pemphigus foliaceus or fogo selvagem (FS) is a blistering autoimmune disease indigenous to certain states of Brazil. In the state of Parana the disease has been reported in the north-central regions where a total of 632 cases were documented in the period of 1940–80. The present study describes a new focus of FS in the western region of the state of Parana. This focus includes a total of 213 new cases of FS and only 11 cases of pemphigus vulgaris seen in this region from February 1976 to July 1988. Over 90% of these patients were peasants working in agriculture or involved in other outdoor activities.

Pemphigus foliaceus or fogo selvagem (FS) is an autoimmune disease reported in Brazil since the beginning of the century.[1] Certain regions of the states of Goias, Mato Grosso do Sul, Parana, Sao Paulo and Minas Gerais show a high incidence of FS. Features which are common to all of these endemic areas are the tropical weather and certain environmental agents such as black flies (*Simulium* spp.) and other insects that may participate in the onset of the disease.[1] FS patients have IgG class autoantibodies, restricted to the IgG4 subclass[2] and directed against desmoglein I (DG-1), a desmosomal glycoprotein.[3] FS autoantibodies induce acantholysis *in vivo*,[2,4] which results in superficial blisters and erosions of the skin (Fig. 1).

Although the non-endemic form of pemphigus foliaceus has been reported sporadically in Paraguay, Argentina, Bolivia and Peru,[5] the endemic form has been reported mainly in Brazil and recently in Colombia.[6] In Brazil, there were more than 15,000 registered cases at the end of 1987[1] clustered in the states of Goias, Mato Grosso do Sul, Parana, Sao Paulo and Minas Gerais.[1]

The state of Parana, where this study was conducted, is bordered by the state of Sao Paulo

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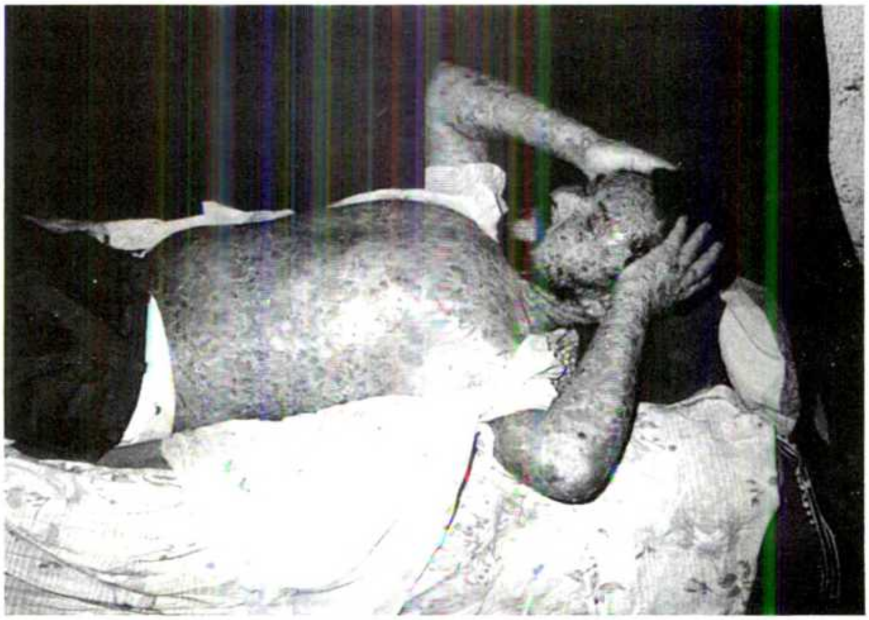

FIGURE 1. This 27-year-old patient from Cascavel county presented generalized superficial erosions with crusting and serum exudate accompanied by systemic symptoms of malaise and fever. Many of the lesions showed secondary bacterial infection. The palms, soles and mucous membranes were the only areas free of lesions.

(north), the state of Santa Catarina (south), the state of Mato Grosso do Sul and Paraguay (west) and the Atlantic Ocean (east). Parana has a total area of 199,554 km^2 and a total population of 8,067,161. The Parana River runs from north to south and forms the western border of the State, where the Itaipu Dam and reservoir is located.

The region defined in this paper as western Parana, corresponds to an area bordered by the Piquiri River to the north, Iguacu River to the south and Itaipu Reservoir to the west (Fig. 2). The weather in this area is hot and humid and the majority of the population is involved in agriculture or outdoor work. The climate and ecology in this region of Parana are quite different from the southern and eastern regions which have a very low incidence of FS.[7-9] It is our observation that the climate and ecology of western Parana have changed since the completion of Itaipu reservoir, becoming more humid and conducive to the propagation of mosquitos and other insects. The population of western Parana is composed of settlers who have migrated to the area over the last 40 years, mainly Caucasians (Germans, Italians, Portugese and Spanish), with a small number of Asians, Blacks, and mulattos. The majority of patients reported by Minnelli[7,8] were from the north-central regions of the state.

The present study, which lasted from February 1976 to July 1988, incorporated a total of 224 new cases of pemphigus. Of these cases, 213 were diagnosed as FS as opposed to only 11 cases of pemphigus vulgaris (PV).

METHODS

The subjects included in this study were seen either in a Community Clinic operated by the Parana State Department of Health or at the private office of one of the authors (J.C.E.), both

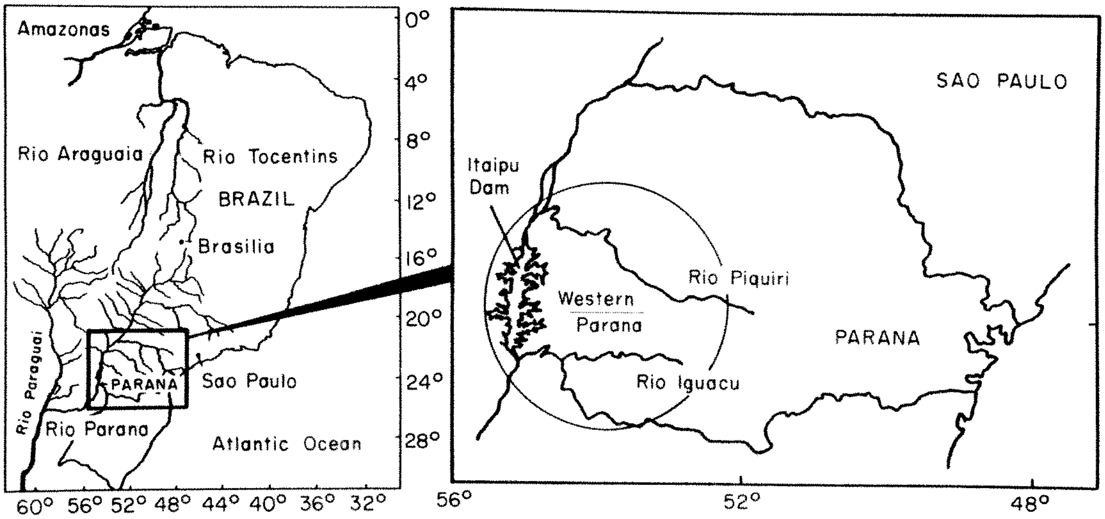

FIGURE 2. The location of the state of Parana in Brazil and, on the right, a higher magnification detailing the area of western Parana; bordered by Piquiri River (north), Iguacu River (south) and Itaipu Reservoir (west) this area represents the newest active endemic focus of fogo selvagem described in Brazil.

located in the city of Cascavel, in western Parana. The study covers the period from February 1976 to July 1988.

Criteria for diagnosis of FS

The diagnosis of FS was based on the dermatological examination of the patients. Only those patients showing the typical cutaneous lesions of FS were included in this study. The diagnosis was confirmed in randomly selected patients by histopathological examination and by direct and indirect immunofluorescence (IF) analysis. Skin biopsies were taken from lesional skin and the IF procedures carried out as described previously.[2,4] Patients entering this study were classified according to the guidelines proposed by the Cooperative Group on Fogo Selvagem Research.[10] A patient from Cascavel county with a classic form of generalized FS is shown in Figure 1.

Epidemiological parameters

Relevant epidemiological data such as race, age, sex, occupation, origin, etc. were obtained from each patient during the first and follow-up interviews according to the epidemiological questionnaire prepared by the Cooperative Group on Fogo Selvagem Research.[10]

RESULTS

Frequency of fogo selvagem and pemphigus vulgaris in western Parana, Brazil

From February 1976 to July 1988 a total of 224 patients presented with a blistering eruption. The disease was labelled as FS in 95·1% of the patients and PV in only 4·9%. The yearly distribution of FS and PV and the clinical forms of FS are shown in Table 1. The county of origin of each of the patients entered in this study is represented graphically in Figure 3 which shows a clustering of patients in the 'municipios' (counties) of Toledo and Cascavel, where 51% of cases occurred.

TABLE 1. Clinical forms of fogo selvagem and pemphigus vulgaris in western Parana (February 1976–July 1988)

Year	Total	Fogo selvagem localized	Fogo selvagem bullous-exfoliative	Fogo selvagem erythrodermic	Pemphigus vulgaris
1976	3	1	0	1	1
1977	6	0	2	3	1
1978	8	5	2	1	0
1979	2	2	0	0	0
1980	14	10	3	1	0
1981	9	3	4	2	0
1982	31	21	6	3	1
1983	21	16	1	2	2
1984	42	22	12	7	1
1985	34	21	8	3	2
1986	24	18	3	2	1
1987	20	10	5	3	2
1988	10	4	5	1	0
Total	224	133 (59·4%)	51 (22·8%)	29 (12·9%)	11 (4·9%)

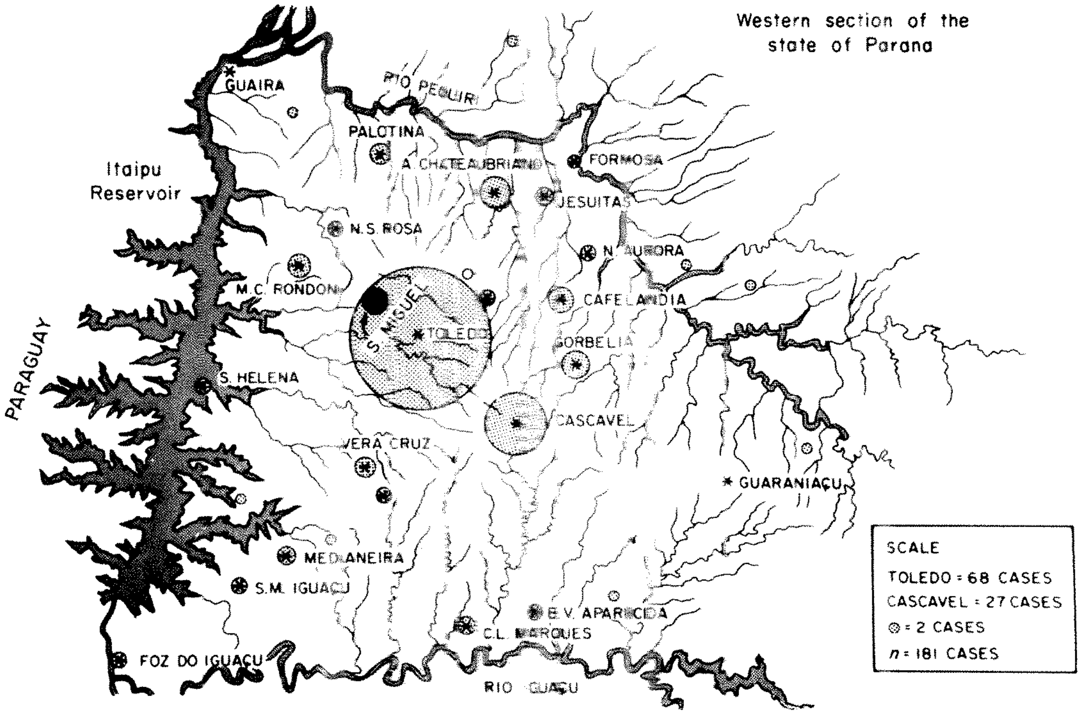

FIGURE 3. Distribution of fogo selvagem patients in western Parana. One-hundred and eighty-one FS patients were seen from January 1976 to May 1987. These patients are registered on the map according to their 'municipio' of origin. The diameters of the shaded circles represent the number of patients (scale shown at the bottom of the figure). Twenty-five new FS patients were diagnosed from June 1987 to July 1988 and are not shown in this figure. Data on these patients are available in Tables 1–4.

TABLE 2. Age distribution of fogo selvagem and pemphigus vulgaris patients in western Parana

Age group (years)	Fogo selvagem	Pemphigus vulgaris
0–9	3	1
10–19	24	0
20–29	55	2
30–39	46	2
40–49	36	4
50–59	23	1
> 60	26	1
Total	213	11

TABLE 3. Distribution of fogo selvagem and pemphigus vulgaris patients by sex, race and marital status

Sex		Race		Marital status	
Males	92	White	217*	Single	55
Females	132	Asian	1	Married	161
		Mulatto	4	Widower	8
		Black	2		

* Brazilian, German, Portuguese, Spanish.

TABLE 4. Distribution of fogo selvagem and pemphigus vulgaris patients by occupation

Profession	Fogo selvagem	Pemphigus vulgaris
Housewife and farmer	74	3
Farmer	64	4
Housewife only (farm)	40	3
Children	1	1
Student	9	0
Other (*)	25	0
Total	213	11

* Fisherman, teacher, missionary, engineer, taxi driver, etc.

Distribution of fogo selvagem and pemphigus vulgaris by age, sex and occupation

Over 50% of the patients were between the ages of 20 and 50 (Table 2) and FS and PV were seen more frequently in females and Caucasians (Table 3). Table 4 shows that most patients were farmers or individuals occupied in outdoor activities.

An increasing number of patients singled out exposure to black flies ('borrachudos') as the precipitating factor of their disease. Since most patients live in houses with poor sanitation, it was difficult to segregate this factor from several other potential environmental aetiological agents (antigens or vectors) that may trigger the disease.

DISCUSSION

From 1941 to 1950, sporadic cases of fogo selvagem (FS) were reported in the State of Parana, with a gradual increase in the number of cases detected in the following years.[9] Minelli[7] reported a total of 506 cases in the same state from 1941 to 1973, and later reported a total of 632 patients seen in Parana from 1940 to 1980.[8] The majority of the patients were clustered in the northern region of the state immediately south of the Paranapanema River, where the weather is hot and humid.

In the late 1930s and 1940s new human settlements were established in western Parana including the study areas. The majority of these settlers were of European background arriving from southern Brazil. Defoliation of the native vegetation to expand agriculture was common. The construction of the Itaipu Reservoir which appears to have modified the climatic conditions of western Parana, and the influx of a genetically homogeneous human population to this area are important factors in the epidemiology of this new focus of FS. From a total of 224 patients with blistering disorders examined in our clinical facilities, 95% were diagnosed as FS and only 5% as pemphigus vulgaris (PV). The apparent difference in frequency of these two autoimmune diseases in western Parana suggests that they may be precipitated by different aetiological factors. PV occurs world-wide in certain genetically predisposed individuals,[11] whereas FS is endemic to particular areas of South America, i.e. Brazil and Colombia.

Confirming the results of other Brazilian investigators[7,8,12,13] we found that patients with FS were poor peasants, farmers and outdoor labourers in rural areas, and usually young. In contrast to previous studies, this investigation showed three unique features of FS in western Parana: a higher frequency of cases in females, an increased rate of localized forms of FS and a predominance of the disease in Caucasians.

It is possible that predisposed Caucasian individuals, which represent the majority of the population in this region of Parana, have a tendency to develop this particular localized form of FS when exposed to the same environmental factors which precipitate the generalized form in other populations. Also, affected individuals in this study may have sought dermatological care early while the disease was still localized while the generalized cases may have migrated to nearby cities such as Campo Grande (state of Mato Grosso do Sul), Londrina or Curitiba (Parana) where hospital facilities exist.

HLA studies in FS patients have recently been reported.[14,15] In a group of FS patients ($n = 24$) 58% were DR1 positive while only 20% of the control group expressed this marker, conferring a relative risk of 5·5.[14] HLA-DR4 was found in 42% of the patients vs. 17% of the control group, and HLA-DR1 and DR4 antigens were found in 83% of the FS patients. HLA-DR7 was present in 17% of the controls but was absent in all FS patients studied, suggesting that this antigen may confer resistance to the development of FS. These findings have been confirmed by other investigators who found an increased frequency of HLA-DR1 and -DR4 phenotypes in a group of 48 patients (88%) as compared to only 34% of the matched-control group ($n = 74$).[15] They also found absence of the DR7 antigen among the FS patients studied.

The ecology and climate in western Parana are similar to those in other regions of Brazil where FS is endemic.[1,7–8,12,13] FS patients in western Parana were exposed to a variety of potential aetiological agents before the onset of their disease. The present study provides further circumstantial evidence that bites of the *Simulium* fly have a role in the onset of FS.

As seen in Table 1 and Figure 3, most of the patients entering our study came from 31 different counties ('municipios') of western Parana. However, the counties with the greatest frequency of cases were Toledo and Cascavel with 52·6% of the total. We have selected Sao Miguel, a small village in the county of Toledo, to carry out a prospective sero-epidemiological study (unpublished). This village has a population of 400, among which eight cases of FS have been diagnosed to date. A long-term follow-up of this population may give us some important clues regarding the aetiology of this endemic autoimmune disease.

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The association of bullous pemphigoid and malignant disease: a case control study

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SUMMARY

In a case control study, the incidence of malignant disease in 84 patients with bullous pemphigoid (BP) was compared with 168 controls. The rate of malignant disease (past, concurrent or during follow-up) in BP patients was 17·9% compared to 5·3% in the controls. A number of the malignancies occurring in the BP group may be of doubtful significance, being either temporally very remote or partially attributable to treatment. The rate of concurrent BP and malignancy (within 8 weeks) was 6·0% suggesting that there is probably a slight excess of malignancy in BP, but insufficient to warrant extensive investigation in pursuit of cancer. Comparison of the BP patients with and without cancer identified no clinical or immunopathological subgroups in whom investigations would be indicated. Three patients with both BP and malignancy were HLA-DR 13 positive, which may point to an immunogenetic predisposition to both diseases.

Bullous pemphigoid (BP) has been reported in association with carcinoma at many different sites[1–14] as well as with sarcoma,[15] melanoma,[16] and lymphoproliferative disorders.[9,17,18] The case for a genuine association is strengthened in a few of these reports by a parallel clinical course between the neoplasm and the blistering eruption,[4,19] though there are also cases where this has not occurred.[9] In other reports the BP has had unusual clinical features, presenting as a figurate erythema[8–19] and has led to the suggestion that atypical BP may be a marker for malignancy.

Despite these numerous reports it remains controversial whether BP and malignant disease are truly associated, or whether their concurrence is merely a reflection of the fact that both diseases are most common in the elderly. There has been only one case control study,[1] and this concluded that there was no higher incidence of malignant disease in BP patients than in age- and sex-matched controls. In contrast, a higher than expected incidence of malignancy was found in BP patients in a study based on population malignancy rates.[20] Of the other published series, some[21–23] but not all[24] have reported a higher incidence of malignant disease in BP

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patients with negative indirect immunofluorescence (IMF) findings compared to those with positive findings, suggesting that seronegative BP may be a marker for internal malignancy.

In this study of 84 patients with bullous pemphigoid, the incidence of malignant disease was compared to that in 168 controls. The clinical and IMF findings and the HLA type in patients with and without malignant disease were analysed in order to identify any BP subgroups at increased risk of malignancy.

METHODS

Bullous pemphigoid patients

We reviewed all 84 bullous pemphigoid patients seen from 1975–89, and in all cases the diagnosis was confirmed by the presence of a linear band of IgG and/or C3 at the dermo-epidermal junction on direct immunofluorescence. The patients' details are shown in Table 1. Special note was made of the indirect IMF findings, the presence of mucosal involvement, milia and any unusual clinical features of the BP. Thirty-seven patients had their HLA type determined using a lymphocyte microcytotoxicity assay.[25] All neoplasms occurring in the BP patients were recorded with the date of their diagnosis. Apart from a history and clinical examination no routine investigations in pursuit of cancer were done other than those required for the management of the patients' treatments. Most patients had full blood counts, biochemistry and liver functions test, but no other tests unless indicated. Non-melanoma skin cancers were not included in the study because of the unreliability of patients' histories in respect of these tumours.

Controls

Each patient was matched by age, sex and calendar year of diagnosis to two controls (168 controls). Controls were obtained from the venous leg ulcer clinics and from patients attending minor operating sessions for removal of skin lesions, including basal cell carcinoma but also a variety of benign skin lesions. Details of the medical histories of the controls were obtained by questionnaire if they were still attending the clinic, and otherwise from their hospital and general practitioner records and where relevant from post-mortem reports and death certificates. The controls were followed up from 3 months to 15 years (mean 3 years 1 month).

The statistical analyses used were the Mantel-Haenzel estimate of the odds ratio and the chi-squared test with Yates' correction.

TABLE 1. Details of bullous pemphigoid patients studied

Total number	84
Sex	M 39 F 45
Age	Range 45–94 years Mean 73·9 years
Follow-up	Range 2 weeks–15 years 1 month Mean 3 years 1 month

RESULTS

Overall frequency of neoplasia

Neoplasms recorded among the BP patients were carcinomas of bronchus, breast, bladder, prostate and colon, two lymphomas (one Hodgkin's disease, one cutaneous T-cell lymphoma), a melanoma and a case of monoclonal gammopathy, which though neoplastic, has so far behaved in a benign fashion over 3 years 2 months of follow-up. Sixteen neoplasms were recorded in 15 BP patients (17·9%), compared to nine in the control group (5·4%; odds ratio = 3·63; $P < 0{\cdot}01$) (Table 2). As the smoking history of patients and controls was unknown, the analysis was also done excluding carcinoma of the bronchus. The frequency of non-bronchus neoplasms was 11/84 (13%) in the BP patients, compared to 8/168 (3·5%) in the controls ($P < 0{\cdot}05$).

Temporal relationship between BP and neoplasia

BP patients. In five patients the cancer preceded the onset of BP, with intervals ranging from 1 year 11 months to 29 years (see patients 1–5, Table 3). In two patients, the temporal relationship between the cancer and BP may have been somewhat closer than indicated by the interval between the two diagnoses. In one patient there was relapse of a breast malignancy with metastases 9 months before the appearance of the pemphigoid after a disease-free interval of over 10 years and in one patient, a second malignancy (prostate) was diagnosed at the time he presented with bullous pemphigoid.

In addition to this patient, there were four others who had their neoplasms diagnosed at the time of their presentation with pemphigoid, or within the following 8 weeks, giving an incidence of strictly concurrent tumours of 5/84 (6·0%). In none of the patients did the course of the neoplasm parallel that of the BP.

Six patients had tumours developing after the onset of BP (range 7 months–4 years, mean 2 years 2 months). All were still receiving treatment for their BP at the time the tumour was diagnosed, including three patients treated with combined prednisolone and azathioprine, a combination known to predispose to neoplasia in the renal transplant population.[26] The doses of azathioprine used were 1·0–1·5 mg/kg per day, though one patient (patient 16, Table 3) received

TABLE 2. Neoplasms recorded in BP patients and controls

	BP		Controls
Bronchus	4		1
Breast	5		3
Bladder	1	single patient	1
Prostate	1		
Colon	1		1
Uterus	0		1
Lymphoma	2		0
Monoclonal gammopathy	1		0
Melanoma	1		2
Totals	16 (in 15 patients)		9
	17·9%		5·4%

$P < 0{\cdot}01$.

TABLE 3. Cancers preceding the onset of BP

Cancer	Interval to diagnosis of BP*	Comment
1 Ca breast	−29 years	
2 Ca colon	−22 years	
3 Ca breast	−11 years	Metastatic relapse 9 months prior to Δ of BP
4 Ca breast	−3 years 6 months	On tamoxifen
5 Transitional cell Ca bladder	−1 year 11 months	2nd malignancy (prostate) Δ at time of onset of BP
Ca prostate	0	Neoplasm detected at time of Δ of BP
6 Ca breast	0	
7 Melanoma	0	
8 Monoclonal gammaopathy	0	
9 Ca bronchus	+1 month	
10 Hodgkin's disease	+7 months	On prednisolone and azathioprine
11 Ca breast	+1 year 8 months	On prednisolone
12 Cutaneous T-cell lymphoma	+1 year 11 months	On prednisolone and azathioprine
13 Ca bronchus	+ 2 years	On azathioprine
14 Ca bronchus	+3 years 4 months	On prednisolone and sulphapyridine
15 Ca bronchus	+4 years	On prednisolone and azathioprine

* A minus sign indicates date of Δ of tumour preceded the onset of BP.

a higher dose (2·5 mg/kg) of azathioprine combined with 7·5–15 mg/day of prednisolone until he presented with cutaneous T-cell lymphoma after 1 year 11 months. In view of the effect of treatment we repeated the analysis excluding the three possibly treatment-related malignancies, giving an incidence of 12/84 (14·2%).

Controls. All the neoplasms in the controls preceded the reference time point (i.e. their first presentation to the skin clinic) by 3 years–17 years 11 months (mean 10 years 1 month). None developed concurrently with their presentation or during follow-up.

Comparison of BP with and without malignant disease

To look for an identifiable subgroup with an increased risk of cancer, we compared the 15 patients with a history of malignant disease with the 69 patients without malignancy (Table 4). The only difference was in the sex ratio with a slight excess of males in the cancer group compared to a female preponderance in the non-cancer group. The prevalence of mucosal

TABLE 4. Comparison of BP patients with and without cancer

	Neoplasia-associated BP	BP without neoplasia
No. of patients	15	69
Mean age at onset BP	75·5 years	73 years
Sex ratio F:M	0·79:1	1·5:1
No. (%) with mucosal lesions	7/15 (44%)	28/69 (40·5%)
No. (%) with circulating Ab	8/10 (80%)	31/46 (67%)

TABLE 5. HLA-DR types in seven patients with BP and neoplasia

DR	2	3
DR	11	—
DR	4	13
DR	4	13
DR	3	13
DR	2	4
DR	4	7

involvement, and the presence of a circulating antibody were looked at but the differences between the groups did not reach statistical significance. There were no differences between the neoplasm and non-neoplasm BP patients in respect of any other clinical feature, notably the duration of prodromal itching, the presence of milia and the morphology and distribution of lesions.

HLA typing

Thirty-seven patients were HLA typed for the A, B, C and D loci, and they did not differ from 151 unrelated controls drawn from our local population. Seven of these patients had both BP and a tumour; the HLA type for the A, B and C loci in these patients did not differ significantly from that in BP patients without a tumour. The HLA-DR types in the patients with both BP and a tumour are shown in Table 5. HLA-DR 13 occurred in three of the seven patients with both BP and neoplasia, compared to only 2/28 of the BP patients without neoplasia.

DISCUSSION

Despite the frequently reported occurrence of BP in patients with malignant tumours,[1-19] it remains controversial whether there is a genuine association[20] or just coincidence.[1,9,10] Only Chorzelski *et al.* found an excess incidence of malignancy amongst BP patients,[20] though other groups have suggested that there may be subgroups of BP, with a increased risk of malignancy, such as seronegative[21-23] or clinically atypical BP.[8,19]

In this case control study, malignancy, whether past, concurrent or developing during follow-up occurred more commonly in BP patients (17·9%) than in controls (5·4%). However, there are important difficulties in interpreting these data, particularly in determining which cases of cancer have a valid relationship to BP. Previous authors have varied in their inclusion criteria, some including only tumours strictly concurrent with BP (i.e. within 6 months),[22] and others allowing more relaxed criteria, such as 5 years before or after the onset of BP.[1] Ahmed *et al.* included those tumours developing during follow up of 5–43 months,[24] but in Chorzelski's series the criteria are not specified.[20] Neither of these reports refer to the possible effects of treatment for BP. In view of the increased incidence of neoplasia in immunosuppressed renal transplant recipients,[26] we excluded the three BP patients who developed neoplasms while receiving prednisolone and azathioprine. This reduced the incidence of BP to 12/84 (14%) which is still significantly higher than in the controls.

Like other authors we also excluded temporally remote tumours from the analysis, though the validity of this is less certain and rests on the assumption that the supposed link between BP and neoplasia operates contemporaneously in both diseases. The most commonly proposed model for a causal link between BP and cancer, that of antigenic cross reactivity between tumour and skin,[4] implies concurrence of onset. In our study, exclusion of three tumours preceding the BP by a long period of time reduced the incidence of BP and cancer to 9/84 (10·7%), and exclusion of *all* tumours not strictly concurrent (i.e. within 8 weeks) further reduced this figure to 5/84 (6·0%), a result similar to the 8% incidence reported for tumours developing 6 months either side of the onset of BP.[22] This pick-up rate for tumours at the time of presentation of BP is likely to be similar in other dermatology departments as the patients in the present study were not routinely investigated for cancer. In the control group, all of the cancers preceded the reference time point by a number of years, and none presented within the 8 week-period on either side of their first clinic attendance. Hence, although there appears to be a difference between BP and controls, reflecting an excess incidence of cancer at or around the time of the development of BP, the true magnitude of this difference is unknown. The incidence of cancer in the control group may be an underestimate, as patients experiencing other problems with their health are less likely to present for management of benign skin lesions.

We also considered the possibility of an alternative model for a link between BP and cancer, that of an underlying genetic predisposition to both diseases, a hypothesis consistent with their non-concurrent onset. No HLA differences in respect of the A, B or C loci were detected between cancer–BP patients, non-cancer–BP patients and controls. Though the numbers are small it is of interest that three of the seven patients with both BP and cancer were HLA-DR 13 positive compared to only 9% of our local population. If confirmed, a link between this antigen and cancer-proneness in BP patients would not readily be explicable in terms of the theory that a breakdown in immunosurveillance predisposes to cancer. The DR-13 antigen contributes to the DR-6 phenotype, which in renal transplant recipients has been associated with heightened rather than impaired immune responsiveness.[27,28] However, if this association with DR 13 is borne out and confirmed as a marker for cancer-associated cases of BP when larger number of cases are studied, then reappraisal of our figures with inclusion of temporally remote tumours would be valid, making the excess incidence of tumours in the BP group more highly significant.

Previous authors have cited a parallel course between the two diseases as further evidence in support of a causal link;[4,19] none of the patients in this study had a parallel clinical course. Despite previous reports that cancer-associated BP is commoner in patients with seronegative BP[21–23] or occurs with morphologically atypical BP[8,19] we have been unable to confirm these findings. Comparison of cancer-associated BP with non-cancer-associated BP patients in respect of the lesion morphology and distribution, mucous membrane involvement and immunopathological findings identified no subgroup at increased risk of cancer.

We conclude that the rate of malignant disease (past, concurrent or during follow-up) in BP patients is higher than in controls, though a proportion of these cases may be of doubtful significance. Disallowing doubtful cases reduces the incidence to 6·0%, similar to the overall incidence in controls. There is probably an excess of cancers clustering around the time of onset of BP though the exact magnitude of this increased risk is difficult to estimate. This level of risk does not support the case for extensive investigations in patients presenting with BP as the detection of cancer at presentation is likely to be low. There also does not appear to be any identifiable subgroup of BP for whom extensive investigations would be indicated. Further studies are needed to determine whether cancer-associated BP is linked to HLA-DR phenotype.

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Linear IgA disease of adults: association with lymphoproliferative malignancy and possible role of other triggering factors

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SUMMARY

Seventy patients with linear IgA disease of adults were followed up for a mean of 8·5 years and all malignant diseases in this group were ascertained. There were three cases of lymphoproliferative malignancy, which constituted a significant excess over the 0·2 cases that would be expected by comparison with an age- and sex-matched population using National Cancer Registry statistics. In contrast, the non-lymphoid malignancy rate of 13% is almost identical to the expected 14%. A subgroup of 35 of the adult linear IgA disease patients were assessed with respect to the possible precipitating illnesses or drugs, as well as co-existing medical conditions. Almost one-third of patients described an event that was felt could possibly have triggered the linear IgA disease, the most frequent being non-steroidal anti-inflammatory or antibiotic drug therapy, trauma/burns and upper respiratory tract infections. However, it is difficult to determine how often the preceding event is coincidental, and how often, if at all, it is causal.

Linear IgA disease of adults (LAD) is a subepidermal bullous disease commencing after puberty in which linear IgA deposits are found at the basement membrane zone. Although the eruption is extremely variable, patients typically have some features of both bullous pemphigoid and, to a lesser degree, dermatitis herpetiformis, such that it is difficult to arrive at a confident clinical diagnosis. The presence of annular lesions with a peripheral urticated ring or blistering is a pointer to LAD. The combination of these features with the high incidence of mucosal involvement, the sulphone responsiveness, and the characterization of a 285 kDa target antigen at the basement membrane zone[1] have led to general acceptance that LAD is a specific entity and not just an IgA bullous pemphigoid.

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The aetiology of LAD is unknown. There have been several case reports suggesting an association with malignancy,[2-7] and in some there has been a parallel course between the malignancy and the LAD. There have also been several case reports of apparently drug-induced LAD.[8-11] We have investigated whether a true association with malignancy exists, and whether any other medical condition or drug therapy is commonly associated with LAD.

METHODS

Subjects

A total of 70 adults with LAD were seen at either Oxford or London. The age at onset varied between 13 and 88 years, with peaks in early adult life and between the ages of 55 and 64 (Fig. 1). The patients have now been followed up for between 6 months and 46 years after the onset of symptoms. The mean follow-up is 7 years for the 35 Oxford patients and 10 years for the 35 London patients. The current disease remission rate, as defined by the absence of symptoms when the patient is off all treatment is 63% for the Oxford cases and 12% for the London cases. We feel this difference may be partly explained by the higher proportion of Oxford cases diagnosed prospectively, with less bias from the selective referral of particularly refractory cases. In cases where non-specific pruritic lesions preceded blistering, the former was taken as representing the onset of the LAD. All cases had subepidermal blistering on histology, lacked circulating antibasement-membrane zone IgG and had a prominent IgA band on direct immunofluorescence at the dermo-epidermal junction.

Ascertainment

All malignant diseases occurring in the 70 patients were ascertained by the combination of case-note review and patient questionnaire in a largely retrospective manner. In no patient had investigations been performed towards finding an otherwise symptomless neoplasm.

In addition, specific enquiries were made of the 35 Oxford patients with respect to drug therapy and other illnesses at or just prior to the onset of their LAD, and to past history of other conditions.

Comparison was made with the 1983 Cancer Registry statistics[12] to determine the cumulative

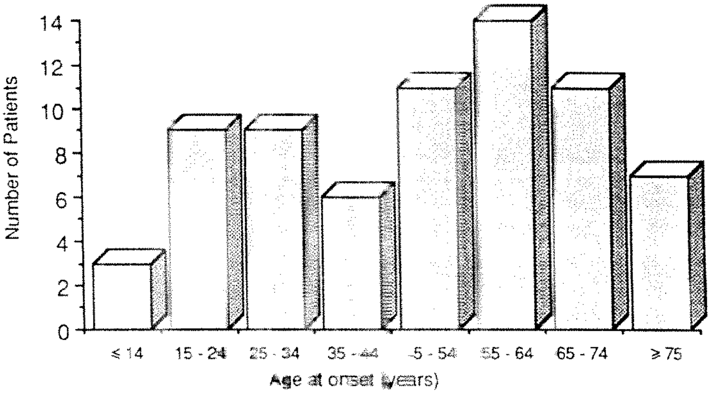

FIGURE 1. Age at onset of linear IgA disease of adults* (41 females and 29 males). *Defined as linear IgA disease beginning after puberty.

life-time age and sex standardized incidence rates of malignancy in the general population. Rates for lymphoproliferative malignancy were taken to include Hodgkin's disease, B- and T-cell lymphoma, lymphocytic leukaemia and myeloma (ICD numbers 200–204). The 1983 figures were used as this approximated to the mid-point of the study. Statistical analysis was carried out using chi-squared analysis with Yates correction.

RESULTS

Non-lymphoid malignancy

There were nine non-lymphoid malignant neoplasms in the 70 patients (Table 1). Single patients had apparently curative surgery for carcinoma of the uterus and a retroperitoneal sarcoma 5 and 2 years, respectively, before the onset of their skin eruption. One patient had a cervical lymph node containing metastatic squamous cell carcinoma removed 4 years before developing LAD; the primary site was not found, and no other tumour is, as yet, apparent. Other patients had a hydatidiform mole,[13] metastatic hypernephroma and an eccrine carcinoma of the skin[14] diagnosed at the time of onset of their skin rash. The last of these patients also had an acoustic neuroma removed 36 years previously. Three patients developed malignancies after the onset of their LAD. In one a malignant melanoma of the eye was noted 8 years later; after enucleation the patient was noted, perhaps coincidentally, to have gone into remission. The other two patients developed carcinoma of the bladder 10 years later, and fatal carcinoma of the colon 7 years later.

Although in a few of these cases there was a temporal relationship between the malignancy and the onset of the skin eruption, the overall malignancy rate of 13% is almost identical to the 14% that would be expected by comparison of an age- and sex-matched population with National Cancer Registry statistics, and no consistent site is involved.

Lymphoproliferative malignancies

Three patients were noted to have lymphoproliferative malignancies, although again no consistent relationship to the LAD was present. In all three cases the tumour was of B-cell origin. From comparison with National Cancer Registry statistics only 0·2 cases of lymphopro-

TABLE 1. Non-lymphoid malignancies occurring in the 70 patients, and their relationship to the onset of the linear IgA disease

Malignancy	Relationship to LAD onset
Carcinoma of the uterus	Preceded LAD by 5 years
Retroperitoneal sarcoma	Preceded LAD by 2 years
Metastatic squamous cell carcinoma	Preceded LAD by 4 years
Hydatidiform mole	Diagnosed at onset of LAD
Metastatic hypernephroma	Diagnosed at onset of LAD
Eccrine carcinoma of the skin	Diagnosed at onset of LAD
Malignant melanoma of the eye	Diagnosed 8 years after LAD onset
Carcinoma of the colon	Diagnosed 7 years after LAD onset
Carcinoma of the bladder	Diagnosed 10 years after LAD onset

liferative malignancy in an age- and sex-matched population would be expected; thus it is extremely unlikely that the association is due to chance, with a P value $< 0{\cdot}001$.

One patient developed LAD lasting 6 months at the age of 29, and then disseminated myelomatosis with an IgG kappa band at the age of 40. After combination chemotherapy the patient achieved remission of his multiple myeloma. The second patient developed LAD at the age of 69 while receiving treatment with chlorambucil for a diffuse lymphoblastic lymphoma. The lymphoma had initially presented 5 years earlier with cervical lymphadenopathy and an IgM kappa band. She died of progressive marrow failure 2 months after the onset of her blistering eruption. The last patient developed fatal centrocytic/centroblastic lymphoma when aged 61. Linear IgA disease had been diagnosed 14 years earlier, since when she had been on continuous treatment with dapsone.

Drug therapy and other precipitating events

Specific enquiry of the 35 Oxford patients revealed that 13 had taken a total of 19 drugs during the month before the onset of their LAD. In seven this was a non-steroidal anti-inflammatory drug (NSAID), generally taken for osteoarthritis. Four patients had taken antibiotics, three diuretics and two beta blockers. Single patients had received treatment with oestrogen, chlorambucil and Co-proxamol.

A close temporal relationship suggesting a possible triggering effect from drugs or other illness was present in 11 of the 35 patients. Five patients had suffered either a bony fracture or cutaneous burns, two severe upper respiratory tract infections treated with antibiotics, and one an untreated severe upper respiratory tract infection. Three other patients had received antibiotics and a NSAID for tetanus, antibiotics for a gynaecological infection and a NSAID for polymyalgia rheumatica, respectively. The disease duration in these 11 patients was similar to that of the group as a whole.

Associated diseases

Assessment of co-existent medical conditions revealed no specific disease associations (Table 2). The prevalences of urticaria and of arthritis are similar to those in the general population. Diseases with an autoimmune basis are, perhaps, slightly over-represented, with two cases each of hypothyroidism, vitiligo and polymyalgia rheumatica, and single cases of autoimmune neutropenia and maturity-onset diabetes.

TABLE 2. Coexisting diseases in the 35 Oxford patients

Disease	Number of patients
Typical 'ordinary' urticaria	3
Undefined sero-negative arthritis	3
Hypothyroidism	2
Vitiligo	2
Polymyalgia rheumatica	2
Ischaemic heart disease	2
Single patients–maturity onset diabetes, autoimmune neutropenia, ulcerative colitis, migraine, hypertension, mitral valve disease and atopy	

DISCUSSION

Our data suggest that a true association between LAD and lymphoproliferative malignancy does exist. It is interesting to compare our data with those of Leonard *et al.* for dermatitis herpetiformis,[15] where an association with lymphoma is widely accepted. They found three cases of lymphoproliferative malignancy in 109 dermatitis herpetiformis patients, as compared with our three cases in 70 LAD patients. A review of the literature reveals three case reports of LAD occurring in association with Hodgkin's disease,[2-4] and one in association with a gastric lymphoma,[5] adding weight to our evidence of a true association with a lymphoproliferative malignancy.

It is not known whether the association results from common aetiological influences, or whether the LAD is simply a paraneoplastic manifestation of the lymphoproliferative disorder. Possible common aetiological influences include either an environmental agent, or perhaps a basic immune defect that cannot as yet be defined. A contribution from confounding variables such as drug therapy cannot be ruled out, but seems unlikely to explain the association. None of our three patients had received azathioprine and steroids, and dapsone therapy is not recognized as being associated with a later risk of lymphoma.

With regard to non-lymphoid malignancy, excluding the individuals included in our current analysis, the literature contains reports of single cases of LAD associated with polycythaemia rubra vera, and carcinomas of the breast,[5] bladder[6] and oesophagus.[7] In none of these was a close or parallel course documented between the LAD and the malignancy. Our data suggest that any association between non-lymphoid malignancy and LAD is more likely to be due to chance.

As far as drug therapy and other medical conditions are concerned, we feel that LAD may possibly be triggered by drugs, trauma/burns or upper respiratory tract infections in a few patients. It is difficult to obtain control data for these events in a sufficiently large age- and sex-matched population to make statistical analysis meaningful, and we did not attempt to do so. In contrast to the two case reports of LAD induced by vancomycin[8] and by diclofenac,[9] none of our patients was rechallenged with the suspected offending drug. Trauma and burns have not previously been associated with LAD, but a triggering effect of trauma has been suggested in some patients with bullous pemphigoid.[16] Upper respiratory tract infection has been tentatively linked with the development of childhood LAD (chronic bullous dermatosis of childhood).[17]

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Chronic urticaria and cancer: an epidemiological study of 1155 patients

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SUMMARY

To evaluate the possible association of malignant disease with chronic urticaria 1155 consecutive cases with chronic urticaria were reviewed. The Swedish Cancer Registry, Stockholm, was searched for records reporting malignancies in the study population (1958–84), and the expected number of malignancies was calculated on the basis of age- and sex-standardized incidence data. A malignancy was diagnosed in 36 patients with urticaria and the expected number of malignancies was 41. In 23 patients the malignancy appeared during the same year as the onset of urticaria or later. The expected number was 25·6. We conclude that chronic urticaria is not statistically associated with malignancy in general.

Many dermatoses have been considered to be related to an underlying internal malignancy.[1] Urticaria and angio-oedema have been reported mainly in association with lymphoproliferative disorders.[2-16] However, not every dermatosis occurring in a patient suffering from malignant disease is causally related. When the dermatosis is common, as with urticaria, the possibility increases that the association has occurred only by chance. We investigated the incidence of cancer in a large number of patients with chronic urticaria and related the observed number of malignancies to the number expected, in a contemporary population with the same age and sex distribution. The incidence of cancers as well as the expected number of cancers were obtained from the Swedish Cancer Registry, Stockholm.

METHODS

Patients

During the years 1968–83, 1155 patients with chronic urticaria were seen at the Department of Dermatology, Karolinska Hospital, Stockholm. All had had symptoms for more than 3 months. The patients consisted of 704 females and 451 males (median age 32 years, age range 1–85 years) (Fig. 1). The follow-up period was on average 8·2 years.

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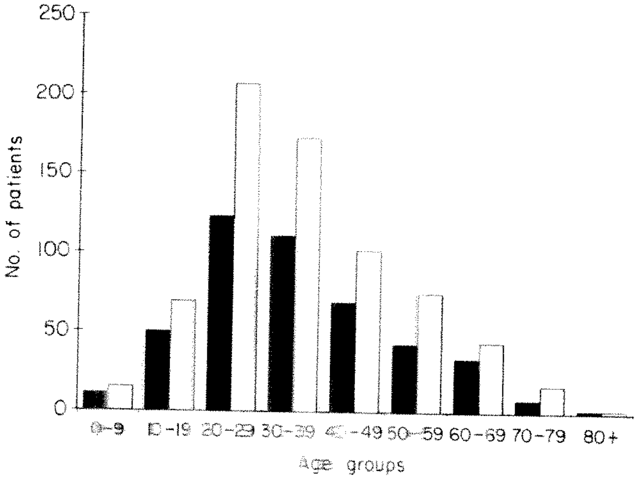

FIGURE 1. Age distribution of 1155 patients with chronic urticaria. ■, males; □, females.

Swedish Cancer Registry

Information from the Swedish Cancer Registry, Stockholm, (1958–84) and the 1155 patients was correlated to identify individuals with malignant tumours. Nationwide information on the cancer incidence in Sweden is available from 1958, when compulsory registration was begun. The registry collects information on diagnosed cancers from both clinicians and pathologists so that most cases are reported by two sources. Each patient is characterized by a unique identification number composed of six digits based on year, month, and day of birth, supplemented with a registration number (three digits) and a check digit. Therefore, the identification numbers are not affected by the possibility of changes in names. The completeness of registration in the Swedish Cancer Registry has been found to be 96–97% for all cancers.[17]

Statistics

The expected number of malignant tumours was estimated on the basis of incidence data from the Swedish Cancer Registry, Stockholm.[18] The age- and sex-specific cancer incidence was calculated as a national average for 1971–84. The incidence rates were assumed to be constant within 5-year age and calendar-time intervals. Regional differences were disregarded. To allow for deaths occurring during the observation period, deductions were made on the basis of life tables for the whole Swedish population. To derive the expected number of malignant tumours, the number of person years at risk in each group was multiplied by the sex-specific and age-specific cancer incidence for the relevant 5-year period. The number of person-years of observation was calculated separately for each sex and amounted to 9400. After the ratio between observed and expected number of malignancies had been estimated, significance and confidence interval analysis was performed using the Poisson distribution.

RESULTS

A malignant tumour was diagnosed in 35 patients with urticaria. The expected number was 41 and thus the relative risk was 0·88, confidence interval 0·61–1·22. Of these patients with malignancy, 23 cancers appeared during the same year as the onset of urticaria or later. The

TABLE 1. Observed and expected number of malignancies in 1155 patients with chronic urticaria

	Number of malignancies	
	Observed	Expected
All patients ($n = 1155$):		
Females ($n = 23$)	23	26·8 NS
Males ($n = 13$)	13	14·2 NS
Total	36	41·0 NS
Cancer the same year as onset of urticaria or later		
Females ($n = 14$)	14	15·7 NS
Males ($n = 9$)	9	9·9 NS
Total	23	25·6 NS

NS, not significant.

TABLE 2. Characteristics of 10 patients with chronic urticaria associated with malignancy (cancer within 5 years after onset of urticaria)

		Age at onset (years) of		
Case	Sex	Urticaria	Malignancy	Type of malignancy
1	M	79	83	Rectum
2	M	69	73	Rectum
3	M	67	71	Colon
4	M	69	72	Stomach
5	M	60	63	Malignant melanoma
6	M	47	52	Skin, squamous cell carcinoma
7	F	61	65	Ovary
8	F	58	60	Corpus uteri
9	F	48	49	Glioblastoma
10	F	78	79	Malignant lymphoma

expected number was 25·6. These differences are not statistically significant (Table 1). Characteristics of those 10 patients who had their malignancy the same year or within 5 years after onset of urticaria are given in Table 2. The study population is shown in Figure 1.

DISCUSSION

This study strongly suggests that chronic urticaria is not statistically associated with malignancy. The study population is well defined and the number of observed and expected cancers are reliable owing to the accurate population statistics used in Sweden.

The link between skin markers and underlying malignant disease has always interested dermatologists. If an association between a skin lesion and internal malignancy has been suggested, the dermatologist has to decide whether there is a need for investigation. If so, how extensive should a search be and how often should the search be repeated? The patient may undergo unnecessary procedures with both socioeconomic and psychological consequences, particularly if the association is false. If the suggested cutaneous sign is common and chronic, because internal malignant diseases are often chronic, it is easy to find patients with both conditions at the same time, as illustrated in Table 2. Therefore, the only way to prove an association of a common skin condition with internal malignancy is to perform a large and controlled study. If, however, the dermatosis is uncommon, e.g. erythema gyratum repens, case reports are the basis for the association as large studies cannot be carried out. We conclude that the hypothesis that urticaria is associated with internal malignant disease was based on case reports only, and this large-scale study has shown that this association probably occurred by chance.

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The effects of an abrasive agent on normal skin and on photoaged skin in comparison with topical tretinoin

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SUMMARY

Two studies were designed to assess the effect of abrasive preparations on the skin and to test the specificity of the effect of topical tretinoin in the management of chronic photodamage to the skin. In the first study two abrasive preparations (Brasivol® fine and Brasivol® medium) were compared with white soft paraffin and no treatment in eight volunteer subjects for their effects on the epidermis. The study was conducted over 3 days and measurements were taken of the effects on dansyl chloride-induced fluorescence to assess desquamation, epidermal thickness, and the tritiated thymidine autoradiographic labelling index. The abrasives were found to increase the desquamation rate significantly and to increase epidermal thickness and the epidermal labelling index compared to white soft paraffin and no treatment.

In the second study the effect of one of the abrasive preparations (Brasivol® medium) was compared with 0·05% tretinoin cream (Retin A®) on the photodamaged skin of the dorsal aspects of the forearms of 12 subjects over an 8-week period. Cutaneous blood flow measured by the laser-Doppler flowmeter was found to be significantly increased in the abrasive-treated sites, but there was only a non-significant trend to increased blood flow in the tretinoin-treated sites. Measurements of skin thickness using pulsed A-scan ultrasound demonstrated that both treatments produced significant increases in thickness over the 8-week period but the increase was greater for the abrasive treated site. Measurements of the skin extensibility at the treated sites were made using a uniaxial extensometer. Forces needed for 30% skin extension were increased in the abrasive-treated sites only. Measurements of epidermal thickness and of [^{3}H]-thymidine autoradiographic labelling indices showed greater increases in the abrasive-treated sites than in tretinoin-treated sites compared to untreated sites, but these increases were not statistically significant.

No significant inflammation and no changes in the degree of elastosis or the presence of a 'repair zone' were found in any of the post-treatment biopsies. The results indicate that some of the changes produced in the skin by topical tretinoin that are taken to indicate a specific

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antiphotoageing effect may not in fact be specific and can be achieved by an abrasive preparation.

Topical tretinoin has been reported to reduce the skin signs of photoageing.[1-3] After 3 months of treatment in a previous study from our department and 4 months in one other, crease lines at the sides of the eyes and the mouth became less prominent, skin colour improved and solar keratoses were reduced in numbers. In addition to these changes, some have claimed that treatment with tretinoin causes a reduction in the telangiectasia, senile lentigines and senile comedones. The mechanism(s) responsible for these beneficial alterations is uncertain, and although there is data concerning the profound effects that retinoid molecules have on gene expression and cell differentiation, there is no direct evidence that it is through these specific mechanisms that topical retinoids exert their clinical action in photoageing. In this study topical tretinoin has been compared with a topical abrasive agent which stimulates desquamation and epidermal proliferative activity via a non-specific mechanical stimulation.

METHODS

Two separate studies were performed.

Study 1. To characterize the epidermal stimulatory and desquamative activity of an abrasive agent

In this part of the study two 'grades' of an abrasive were compared with white soft paraffin and 'no treatment' in a group of eight normal volunteer subjects using a randomized complete-blocks design. The subjects, who gave their informed consent, included two females and six males (mean age 28 years, 20–36 years). The abrasive used was Brasivol®, a soap paste containing particles of aluminium oxide. The 'fine' and 'medium' grades were used.

Two sites, each 3 × 2 cm, were delineated on the lateral aspect of each thigh. To each of three sites on each individual one of the abrasive preparations or white soft paraffin was applied according to a randomization schedule. The fourth site was left untreated. The preparations were rubbed into the skin surface of the allocated areas for 30 s, twice daily for 3 days.

Twenty-four hours before the treatment period began, 5% dansyl chloride in white soft paraffin was applied occlusively in a Finn chamber on Scanpor tape to the superior pole of each test area. At the end of the treatment period, the degree of fluorescence of each site was assessed using a fluorescence comparator.[4] 'Background' fluorescence readings were taken at the same time from a nearby non-dansyl chloride-treated area of skin.

One 4-mm-diameter trephine biopsy was taken from each of the test sites at the end of the treatment period. The biopsies were bisected. One-half was fixed in 10% formaldehyde saline, processed histologically by routine methods, embedded in paraffin and sectioned at 5 μm prior to being studied histometrically in an Optomax image analyser. Mean epidermal thickness (MET) in μm, mean keratinocyte height (MKH) in μm and the mean ratio of the basal-layer length to the granular-cell-layer length (B/G ratio) were determined for each specimen. The other half was divided into smaller fragments and incubated for 4 hours in Eagle's minimal essential medium in the presence of [^{3}H]-thymidine (Radio Chemicals, Amersham), sp.ac. 18 Ci/mmol, 2 μCi/ml. The fragments were fixed and routinely processed histologically and autoradiographically using the dipping-film technique.[5] Labelling indices were determined by identifying basal epidermal and suprabasal cells that had over six silver grains more than background over the cell nucleus and expressing this number as a percentage of the total number of basal cells.[6]

Study 2. To compare the effects of topical tretinoin with that of an abrasive agent on photoaged skin

In the second part of the study 0·05% tretinoin cream (Retin-A® cream) was compared with an abrasive preparation (aluminium oxide particles in a soap paste—Brasivol®, medium grade) in 12 subjects who showed changes of photoageing including wrinkles, a faint sallow yellowish discoloration and senile lentigines. The subjects (four men, eight women; mean age 56 years, age range 49–70 years) were healthy and gave their informed consent to participate in the study. The study design was that of a randomized, open, within-subject comparison of the two materials.

The materials were applied according to the randomization schedules to 8 × 5 cm delineated areas on the extensor aspects of either forearm. The materials were rubbed into the treatment sites for 30 s on each occasion twice daily for 8 weeks.

The following assessments were made: (1) skin blood flow on days 0, 11, 25, 39 and 53, using a laser-Doppler blood flow device[7] (Perimed PF2); (2) skin thickness on days 0, 11, 25, 29 and 53 using a pulsed A-scan ultrasound device[8] (Cutech-Dermal Depth Detector); (3) skin extensibility on days 0, 11, 25, 29 and 53 using a uniaxial extensometer (Cutech) along the long axis of the arm to record the forces generated by extension of two tabs stuck to the skin surface to 30% more than the original 1 cm distance separating them;[9] (4) histometric observations on 4 mm trephine biopsies taken from each of the treated sites and from a third untreated site on the forearm using 2% lignocaine local anaesthesia. The biopsies were halved and as in the first study, the epidermal thickness and labelling indices determined. In addition, the histological sections were assessed for the degree of inflammatory cell infiltrate present using a visual analogue scale scoring system.[10] Measurements were also made (on sections stained with Halmi stain to show elastic tissue) of the thickness of the zone of elastotic degeneration and of the distance from the top of the elastotic zone to the basal layer of the epidermis ('grenz zone' or repair zone).

Statistical analysis

Friedman's non-parametric two-way analysis of variance was used in both studies. The test is performed on two bases, the first null hypothesis being that the values for each separate treatment do not change through the period of treatment. The second null hypothesis states that the differences in the values between the treated sites vary only due to chance. The first test uses raw data from each treatment separately, the second test uses the data from one treatment subtracted from the other for each individual time point. All tests used $P < 0{\cdot}05$ as the criterion for rejecting the null hypothesis. Wilcoxon's matched-pairs signed-ranks test was also used in some instances.

RESULTS

Study 1

Dansyl chloride fluorescence. The individual and group mean values are given in arbitrary fluorescence units less background (Table 1). Multiple comparison showed that the abrasives were significantly different from one another and from the white soft paraffin site and the untreated site ($P < 0{\cdot}01$). White soft paraffin and untreated sites were not statistically different.

TABLE 1. Dansyl chloride fluorescence

Volunteer number	Untreated	White soft paraffin	Abrasive (fine)	Abrasive (medium)
1	55	46	28	14
2	50	52	23	3
3	50	48	8	14
4	50	48	21	17
5	54	50	34	22
6	41	43	17	11
7	70	58	39	18
8	58	50	34	5
Mean	53·5	50·6	25·4	13
SD	8·3	9·5	10·0	6·5

Histometric observations and autoradiographic labelling indices. The individual and mean values for MET, MKH, B/G ratio and labelling indices are given in Table 2. Multiple comparisons between each of the treatment groups showed significant differences ($0{\cdot}01 < P < 0{\cdot}05$), the MET for the abrasive (fine grade) being greater than that for the white soft paraffin and untreated groups. There was no statistical difference between the two grades of abrasive. There were no differences between the treatment groups for MKH and B/G ratio. The labelling indices for the medium grade abrasive were significantly different ($0{\cdot}01 < P < 0{\cdot}05$) from those for the white soft paraffin treated and the untreated sites but not significantly different from that for the fine grade abrasive-treated sites.

Study 2

Skin blood flow. Mean and standard deviations of the cutaneous blood flow over the 8-week period for the two treatments are given in Table 3. Table 4 gives all the relevant statistical significances. In sites treated with the abrasive there was a significant difference between the values obtained at the different time points. Values at time 0 were significantly less than those at all other times and values at 8 weeks were less than those at 6 weeks. No such differences were found in the tretinoin-treated sites and the Wilcoxon's matched-pairs signed-rank test failed to demonstrate a difference between baseline values for each treatment group. However, there was a trend to increased blood flow at the tretinoin-treated sites. There were no significant differences between the treatments.

Skin thickness. The means and standard deviations of the ultrasound-derived values for total skin thickness over the 8-week period for both treatments are included in Table 3 and all the relevant statistical significances are shown in Table 4. The means are also displayed in histogram form (Fig. 1). In the abrasive-treated areas the values at time 0 were significantly less than at 4, 6 and 8 weeks, with that at 4 weeks being less than that at 6 weeks and that at 6 weeks being less than that at 8 weeks. In tretinoin-treated sites the values at 0 and 2 weeks were significantly less than those at 4, 6 and 8 weeks and those at 4 weeks were less than those found at 6 and 8 weeks.

Skin extensibility. The means and standard deviations of the forces generated by the standardized extension over the 8-week period for both treatments are included in Table 3.

TABLE 2. Epidermal measurements at control and abrasive treated sites at the end of the three day treatment period

Volunteer no.	Untreated				White soft paraffin				Abrasive (fine grade)				Abrasive (medium grade)			
	MET	MKH	B/G ratio	LI	MET	MKH	B/G ratio	LI	MET	MKH	B/G ratio	LI	MET	MKH	B/G ratio	LI
1	63·1	11·9	1·21	7·7	59·2	12·1	1·06	4·5	72·3	11·6	1·44	5·3	64·4	11·7	1·11	9·7
2	58·0	12·8	1·03	5·0	62·5	12·5	1·01	12·7	70·5	13·4	1·01	15·6	75·6	13·2	1·10	10·0
3	72·8	12·2	1·24	4·3	81·5	12·4	1·33	5·0	90·0	11·1	1·30	13·2	72·1	12·6	1·11	14·6
4	68·0	11·6	1·03	6·0	78·3	13·0	1·01	5·4	63·2	10·6	1·05	9·5	81·0	11·4	1·02	14·6
5	62·4	11·7	1·16	5·8	62·0	11·4	1·02	16·6	92·4	11·9	1·18	15·7	80·4	11·8	1·03	22·5
6	56·2	11·35	1·01	14·5	62·9	11·01	1·11	7·4	95·4	14·48	1·21	40·4	84·3	13·22	1·03	33·2
7	79·5	11·96	1·35	9·9	69·5	11·08	1·24	14·3	72·7	11·19	1·40	6·4	69·7	10·4	1·49	10·4
8	76·0	12·2	1·02	20·5	60·8	10·4	1·11	23·2	87·8	12·2	1·27	8·5	80·1	12·0	1·06	29·5
Mean	67·0	11·96	1·13	9·21	67·1	11·7	1·11	11·14	80·5	12·06	1·23	14·33	75·95	12·04	1·12	18·06
SD	8·5	0·5	0·13	5·6	8·5	0·9	0·1	6·7	12·1	1·3	0·15	11·3	6·8	0·95	0·15	9·2

MET, Mean epidermal thickness (μm).
MKH, Mean keratinocyte height (μm).
B/G ratio, Basal layer length to granular layer length.
LI, Labelling index (percentage).

TABLE 3. Results (means ± SD) of 8-week study comparing abrasive treatment with 0·05% tretinoin—skin blood flow, skin thickness and skin extensibility

Time (weeks)	Laser-Doppler flowmetry (volts)		Extensometry data (g force)		Ultrasound skin thickness (μs)	
	Abrasive	Tretinoin	Abrasive	Tretinoin	Abrasive	Tretinoin
0	0·15 ± 0·15	0·18 ± 0·19	393·33 ± 144·53	341·67 ± 156·84	1·39 ± 0·14	1·41 ± 0·14
2	0·23 ± 0·11	0·19 ± 0·17	643·33 ± 329·98	463·33 ± 220·28	1·42 ± 0·15	1·44 ± 0·16
4	0·28 ± 0·19	0·22 ± 0·18	833·33 ± 378·54	415·83 ± 174·38	1·47 ± 0·15	1·49 ± 0·16
6	0·40 ± 0·47	0·36 ± 0·34	757·50 ± 304·97	447·50 ± 261·86	1·52 ± 0·20	1·55 ± 0·23
8	0·22 ± 0·19	0·35 ± 0·57	976·67 ± 302·06	520·83 ± 265·47	1·59 ± 0·21	1·55 ± 0·19

TABLE 4. Summary of statistical analyses

	Tretinoin	Abrasive	Tretinoin vs. abrasive
Extensometry	$P > 0.05$	$P < 0.05$	$P < 0.05$
Time	0, 6, 4, 2, 8*	0, 2, 6, 4, 8	0, 2, 6, 4, 8
Laser-Doppler	$P > 0.05$	$P < 0.05$	$P > 0.05$
Time	0, 2, 4, 8, 6	0, 8, 4, 2, 6	8, 0, 6, 4, 2
Ultrasound	$P < 0.05$	$P < 0.05$	$P > 0.05$
Time	0, 2, 4, 6, 8	0, 2, 4, 6, 8	0, 4, 6, 2, 8

* The differences between treatments/times were assessed as described in the text. The underlining convention used in the table above indicates where statistical significance exists. Any two (or more) experimental groups connected by underlining are *not* significantly different at the probability value indicated.

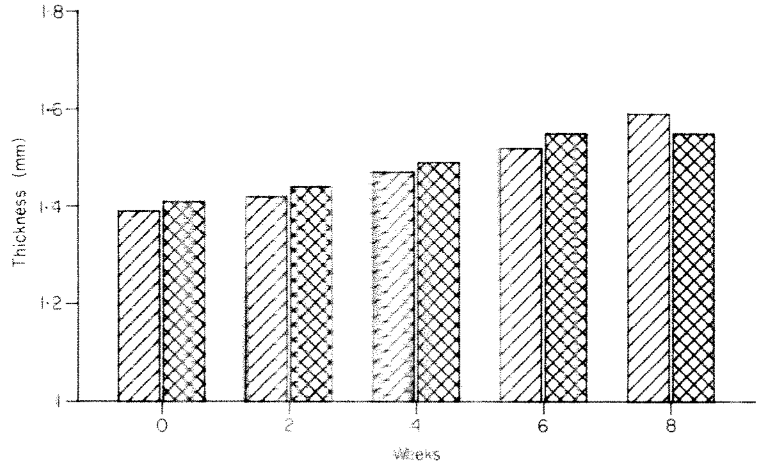

FIGURE 1. Diagram to compare the effects of treatment with tretinoin or an abrasive preparation on skin thickness over a period of 8 weeks, using pulsed A-scan ultrasound. Mean values, $n = 12$. ▨, abrasive; ▩, tretinoin.

Table 4 gives the relevant statistical significances. The results are also displayed in graphic form in Figure 2. Values for abrasive-treated sites at time 0 were significantly less than at all other times and the values at 2 weeks are also different to those at other time points, as is the difference between the values at 6 and 8 weeks. There were no significant differences in the tretinoin-treated sites. The Wilcoxon's matched-pairs signed-rank test indicated that the apparent lack of effect of tretinoin was not due to a significant difference in the initial values. There were significant differences between treatments at 4, 6 and 8 weeks.

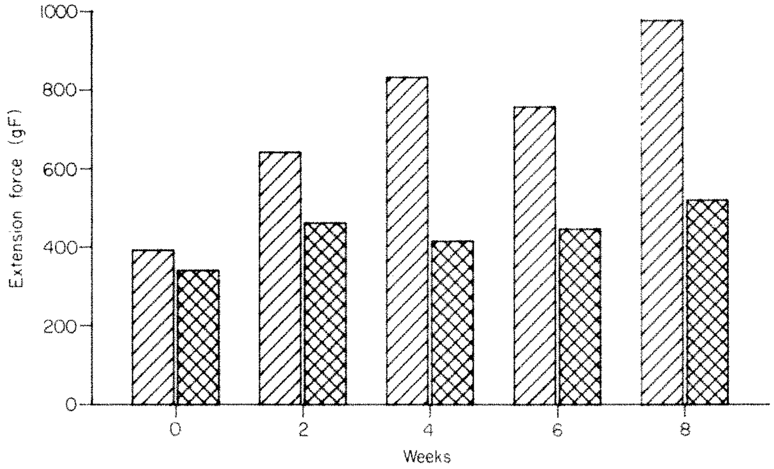

FIGURE 2. Diagram to compare the effects of treatment with tretinoin or an abrasive preparation on skin extensibility over a period of 8 weeks, using an extensometer. Mean values, $n = 12$. ▨, abrasive, ▩, tretinoin.

TABLE 5. Histometric observations

Parameter (unit)	Abrasive-treated site	Tretinoin-treated site	Untreated site
Epidermal thickness (μm)			
Mean	71·80	68·39	63·49
SD	9·10	13·80	9·24
Labelling index (%)			
Mean	11·1	9·4	6·8
SD	3·7	3·5	2·2
Thickness of elastotic tissue (μm)			
Mean	287	355	290
SD	97	184	138
Thickness of grenz (repair) zone (μm)			
Mean	43	41	38
SD	19	16	23
Degree of inflammatory cell infiltrate (cm)*			
Mean	1·17	2·33	0·58
SD	1·57	4·21	0·64

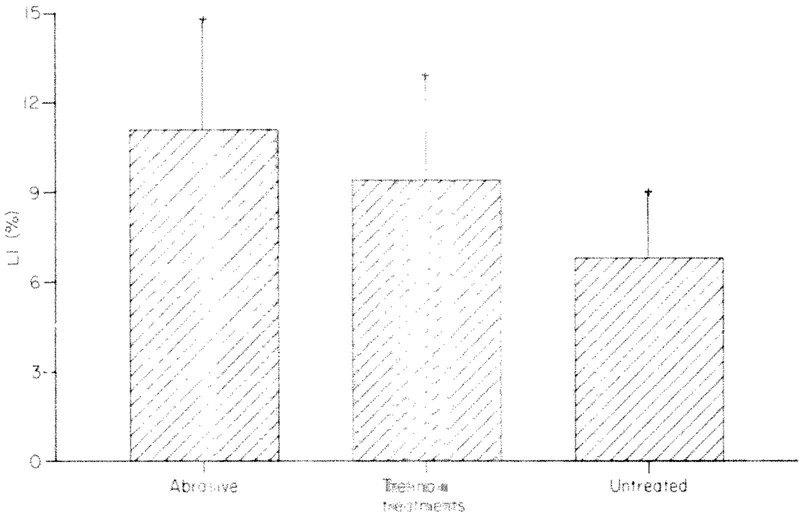

FIGURE 3. Diagram to show the effects on the autoradiographic labelling index after 8 weeks treatment with tretinoin or an abrasive preparation compared to normal untreated skin. Mean values + 1 SD ($n = 12$).

Histometric observations. The means and standard deviations for epidermal thickness, labelling indices, the degree of inflammation present and the thicknesses of the elastotic zone and the 'grenz' or repair zone above the elastotic tissue are given in Table 5. The differences between the means for epidermal thickness and labelling index (Fig. 3) are in the rank order: abrasive-treated sites > tretinoin-treated sites > untreated sites, but fail to reach statistical significance at the 5% level.

DISCUSSION

The first and shorter study demonstrated that a material containing abrasive particles when rubbed on to the skin can stimulate desquamation and epidermal proliferation. The 'dose-effect' relationship for the two grades of the abrasive suggests that the stimulatory effect is the result of the mechanical trauma from the particulate material. The coarser the grains the greater the injury. The increases in epidermal thickness and epidermal labelling index in the second and longer study just failed to reach statistical significance, which may be due to a degree of accommodation being reached to the mechanical stimulus.

Pulsed A-scan ultrasound measurements demonstrated an increase at the tretinoin treated sites as we have found previously.[3] There was an even greater increase in total skin thickness at the abrasive-treated skin sites. We have no evidence that the increase in skin thickness is due either to an increase in the synthesis of dermal connective tissue or to inflammation and oedema. Histologically, the only alterations detected at the two sites treated with the tretinoin preparation and the abrasive respectively are related to the epidermal thickening. It is known that in UV-irradiated mice there is an increase in thickness in the subepidermal repair zone after treatment with topical retinoic acid.[11,12] The failure to detect evidence of increased connective tissue synthesis in man after treatments with retinoic acid[2,3] may be a reflection of the short treatment period in comparison with the animal studies. Furthermore, the increase in skin thickness detected in the human studies using ultrasound may be due to an increase in soluble dermal components such as proteoglycans, which would not have been detected histologically.

The increased resistance to extension noted at the abrasive-treated sites but not at the tretinoin-treated sites reflects an alteration in the mechanical properties of the dermis, but whether this is the result of a change in the dermal connective tissue structure or increased quantities of some dermal component is not clear. The increased skin thickness detected may indicate that the latter explanation is the more likely.

The 8-week study also demonstrated that there was an increase in the skin blood flow at the abrasive-treated sites but not at the tretinoin-treated sites. The lack of an effect of tretinoin on cutaneous blood supply in this study was disappointing in view of the apparent stimulatory effect of this drug on angiogenesis,[13,14] and may have been due to the comparatively short time of treatment. In a previous study we found that tretinoin increased the von Willebrand factor antigen content of skin, suggesting that the drug stimulated endothelial cell proliferation.[15] The effect of the abrasive on blood supply reported here is clearly a result of the mechanical stimulation and it is not known whether this was due to neoangiogenesis or to vasodilatation.

Our study shows that an abrasive can have similar and even more dramatic effects on the parameters chosen to judge the antiphotoageing effects of topical retinoids. It is important to determine the clinical effects of an abrasive in comparison with topical retinoin, and such studies are now in progress. However, the results obtained here suggests that some of the alterations noted with topical tretinoin such as the epidermal thickening, the skin thickening and the increase in blood supply may not be specific to topically applied retinoids.

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Fibroblast–keratinocyte interactions in psoriasis: failure of psoriatic fibroblasts to stimulate keratinocyte proliferation *in vitro*

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SUMMARY

We have tested in two ways the hypothesis that dermal fibroblasts direct the hyperproliferation of the overlying epidermis in psoriasis. First, culture medium from psoriatic and from normal skin fibroblasts was added to monolayer cultures of foreskin keratinocytes. Second, psoriatic and normal fibroblasts embedded in hydrated collagen lattices were co-cultured with monolayers of foreskin keratinocytes. There was no evidence in either study that psoriatic fibroblast products could stimulate the proliferation of the keratinocytes, or that normal fibroblast products inhibited their proliferation. A positive control for the fibroblasts was provided by leucocyte supernatants, which stimulated keratinocyte proliferation by up to 65%. Our data do not support a primary role for dermal fibroblasts in psoriasis.

In 1983 we demonstrated abnormal proliferation rates, protein synthesis, glycosaminoglycans secretion and serum dependence in fibroblasts cultured from both involved and uninvolved psoriatic skin.[1,2] This has since been consolidated by reports of more fibroblast abnormalities by other groups.[3-5] There have also been several attempts to investigate the possibility that the fibroblast abnormalities are the primary defect in psoriasis, and that psoriatic fibroblasts can induce hyperproliferation in the overlying but otherwise normal epidermis. Saiag and his colleagues[6] reported that psoriatic fibroblasts grown in a collagen lattice, or so-called dermal equivalent, could influence the outgrowth of keratinocytes from explants of whole normal skin embedded in the lattice surface. They suggested that soluble factors released by the fibroblasts were responsible for this effect. We have re-examined this hypothesis by studying the interaction of psoriatic fibroblasts with normal keratinocytes *in vitro*.

METHODS

Keratinocytes were cultured from normal foreskins obtained by infant circumcision. Epidermis and dermis were separated in dispase (protease type IX; Sigma Chemical Co., Poole, Dorset) at

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4°C overnight and the epidermis was incubated in 0·25% trypsin in phosphate-buffered saline (PBS) at 37° for 30 min to release epidermal cells. The cells were collected by centrifugation, counted and plated in 24-well or 12-well plastic dishes (Nunc) in Dulbecco's modified Eagle's medium (DMEM) diluted 3:1 with Ham's F12 medium and containing 10% fetal calf serum, penicillin, streptomycin, 10^{-10}M cholera toxin (Sigma), 5 μg/ml insulin, 5 μg/ml transferrin and 2×10^{-9}M tri-iodothyronine (Sigma). The plating densities of the crude epidermal cell suspension were approximately 7×10^5 cells per well in 24-well dishes and $1{\cdot}5 \times 10^6$ cells per well in 12-well dishes. The dishes were incubated at 37°C in 5% carbon dioxide: 95% air. After 2 days the culture medium was changed and supplemented with 10 ng/ml epidermal growth factor. The culture media and supplements were obtained from Gibco (Paisley, Scotland) unless specified otherwise.

Fibroblasts were recovered from liquid nitrogen storage, having been grown from the lesional forearm skin of patients with generalized plaque psoriasis (PSA cell lines) or from the normal forearm skin of healthy individuals of similar ages (HSF cell lines) in previous studies.[1,7] Stock cultures were maintained in 25 or 75 cm² plastic flasks flushed with 5% CO_2 in air at 37°. The medium, DMEM with 10% fetal calf serum, penicillin and streptomycin, was changed three times per week. Cell counts were carried out in a Coulter counter using cell suspensions prepared with trypsin–versene (fibroblasts) or 1:250 trypsin (keratinocytes).

Conditioned medium experiments

For each experiment a pair of fibroblast cultures, one psoriatic (PSA) and one normal (HSF), were grown to confluence in 25 cm² flasks, representing donors of similar age and cell lines of similar passage number (4–9). Medium added 2 days previously was removed, centrifuged to remove any loose cells, and the supernatants were fed to subconfluent keratinocyte monolayers in a 12-well dish. Four wells received medium which had been pre-incubated without cells, four wells received PSA-conditioned medium and four received HSF-conditioned medium (1·5 ml per well). Two days later all the wells were washed with PBS, trypsinized and the cells were counted. Six experiments were performed, each with a different pair of fibroblast lines and keratinocytes cultured from different donors.

Keratinocyte-fibroblast lattice co-cultures

Collagen lattices were made on the permeable polycarbonate membranes of plastic inserts (Nucell, from Sterilin) designed for 24-well dishes. Each insert has a flange which allows it to be suspended in the well without touching the bottom. A solution of rat-tail tendon collagen was mixed with double-strength fibroblast culture medium and fibroblast suspension at 4° and 0·4 ml of the mixture was added to each insert.[8] A brief incubation at 37° caused the mixture to set, giving a lattice with 2×10^4 fibroblasts and a final collagen concentration of 1·4 mg per ml. In each 24-well dish, eight lattices prepared without fibroblasts served as blanks, while eight lattices contained PSA fibroblasts and eight contained HSF fibroblasts. Different pairs of fibroblast lines were used in each of six experiments. The lattices were cultured with subconfluent monolayers of keratinocytes growing in the bottom of the wells for 2 days. The lattices were then removed and the keratinocytes washed and trypsinized for counting.

Effect of leucocyte supernatants on keratinocytes

The supernatants had been prepared for previous studies[9] and stored at −20°. Peripheral blood collected from 12 psoriatic patients and 12 age-matched controls was fractionated into mononuclear and polymorphonuclear leucocytes by centrifugation on Mono/poly resolving medium (Flow Labs, Irvine, Scotland). Leucocytes from different individuals were cultured separately for 3 days in RPMI 1640 medium containing 10% fetal calf serum. Supernatants were then pooled to give four preparations: normal mononuclear and normal polymorphonuclear supernatants and psoriatic mononuclear and psoriatic polymorphonuclear supernatants. Each pooled sample was diluted 1:1 with fresh keratinocyte medium (DMEM-F12) and fed to growing keratinocyte monolayers in a 24-well dish. At that time four untreated cultures were trypsinized and counted to provide baseline cell counts. Four cultures received blank medium (fresh keratinocyte medium plus pre-incubated RPMI 1640 with 10% fetal calf serum). The treated cultures were trypsinized for cell counts 3 days later and proliferation in each was calculated by subtracting the mean baseline cell count.

RESULTS

In the six experiments in which keratinocytes received culture medium conditioned by normal or psoriatic fibroblasts, there was a slight tendency for higher keratinocyte counts with conditioned media than with the unused media (Table 1). In only one experiment (KC38) was

TABLE 1. Proliferation of normal human keratinocytes fed fibroblast-conditioned medium. Cell numbers (10^5) per well ± SEM; $n = 4$ (% change)

Experiment	Blank	HSF	PSA
KC11	1·9 ± 0·1	2·1 ± 0·2 (+5)	1·9 ± 0·1 (0)
KC13	4·1 ± 0·2	5·4 ± 0·5 (+32)	4·3 ± 0·9 (+5)
KC15	2·2 ± 0·2	2·3 ± 0·1 (+5)	2·1 ± 0·1 (−5)
KC21	5·0 + 0·2	5·7 + 0·2 (+14)	5·1 ± 0·1 (+2)
KC31	5·8 ± 0·3	7·0 ± 0·3 (+21)	5·5 ± 0·2 (−5)
KC38	5·8 ± 0·2	6·7 ± 0·1 (+16)	8·1 ± 0·1 (+40)
Means	4·1 ± 0·7	4·9 ± 0·9 (+20)	4·5 ± 1·0 (+10)

Paired *t*-tests: HSF vs. Blank $P < 0{\cdot}02$; HSF vs. PSA NS $P > 0{\cdot}1$; PSA vs. Blank NS $P > 0{\cdot}1$.

TABLE 2. Keratinocyte proliferation with fibroblast-populated lattices. Cell numbers (10^5 means ± SEM; $n = 8$)

Experiment	Blank lattice	HSF lattice	PSA lattice
1	2·53 ± 0·13	2·65 ± 0·12	1·64 ± 0·13*
2	1·84 ± 0·29	1·67 ± 0·24	1·28 ± 0·14*
3	3·60 ± 0·13	3·77 ± 0·14	3·69 ± 0·22
4	4·34 ± 0·23	4·86 ± 0·21	5·14 ± 0·06
5	1·54 ± 0·05	1·54 ± 0·06	1·56 ± 0·08
6	1·25 ± 0·07	1·01 ± 0·03	1·06 ± 0·03
Relative overall means	100	99 ± 5	91 ± 9

* *t*-test: PSA vs. Blank $P < 0{\cdot}001$.

TABLE 3. Proliferation of normal human keratinocytes fed normal or psoriatic leucocyte supernatants

	(i)	(ii)	Pooled mean
Blank medium	100 ± 1[illegible]	100 ± 22	100 ± 12
Normal MNS	129 ± 1[illegible]	165 ± 11	147 ± 10*
Normal PMNS	100 ± 2[illegible]	150 ± 12	125 ± 15
Psoriatic MNS	145 ± 9	159 ± 8	152 ± 6**
Psoriatic PMNS	97 ± 6	158 ± 19	128 ± 15

Mean values are shown ± SEM ($n = 4$) $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$ vs. controls (Wilcoxon test).

MNS, mononuclear leucocyte supernatants.

PMNS, polymorphonuclear leucocyte supernatants.

there a differential effect favouring the PSA-conditioned media, and the overall mean totals for cultures with the PSA- and HSF-conditioned media were not significantly different.

Table 2 shows a similar lack of differential effect between PSA and HSF skin fibroblasts growing in collagen lattices in co-cultures with keratinocytes. An apparent inhibition by the PSA-fibroblasts in the first two experiments was absent in other experiments.

In contrast, keratinocyte proliferation was stimulated by the leucocyte supernatants (Table 3). The mononuclear supernatants were effective in both experiments, but the polymorphonuclear supernatants were only effective in the second experiment. In overall terms there was no difference in the effects of supernatants from the blood of normal and psoriatic subjects.

DISCUSSION

The abnormality of psoriatic skin fibroblasts derived both from lesions and from perilesional skin is now well established. Following our original observations,[1,2] we reported differential sensitivities to clobetasol propionate[10] and to retinoids[7] in the psoriatic cell lines. Abnormal responses by psoriatic fibroblasts[7] to 1,25-dihydroxyvitamin D_3,[3] to platelet-derived growth factor,[4] and deficiency of cyclic AMP-dependent protein kinases,[5] have also been discovered and the occurrence of this last defect in erythrocytes also rules out an *in vitro* artefact.[11] There is abundant evidence of dermal abnormalities *in vitro*.[1]

It has yet to be proved, however, that the fibroblast abnormality is the primary defect in the skin. There are two alternative hypotheses: that the epidermal hyperplasia is primary and subsequently affects the dermis; and that a systemic stimulus is transmitted via the blood. The main evidence that fibroblasts initiate epidermal hyperplasia is the report by Saiag *et al*,[6] whose findings suggest either that psoriatic fibroblasts stimulate keratinocyte growth *in vitro*, or that normal fibroblasts suppress keratinocyte growth. In earlier attempts to answer this question, psoriatic fibroblasts were used as feeder layers for normal keratinocytes.[12] In our laboratory, medium from psoriatic fibroblasts was transferred to cultures of NCTC 2544 epithelial cells, originally derived from human skin,[13] and Otto and Dover injected mixtures of psoriatic fibroblasts and normal keratinocytes into the skin of nude mice.[14] None of these studies showed a differential stimulation by the psoriatic fibroblasts.

In the present study we transferred fibroblast-conditioned media to human keratinocytes. Normal and psoriatic skin fibroblasts vary considerably[1,2] and it was important to use a range of cell lines from each group. In each experiment blank-treated cultures were included for comparison so that inhibitory effects would also be apparent. The use of feeder layers, usually lethally-irradiated 3T3 mouse fibroblasts, for the keratinocytes was avoided. The results showed that the keratinocytes were neither stimulated by the psoriatic fibroblasts nor inhibited by the normal fibroblasts. Comparisons were also made using collagen lattices, since fibroblasts grown in such three-dimensional matrices can behave more like those in the skin than cells in fibroblast monolayers.[15] Specialized fibroblast products might therefore be more likely to appear in the lattice cultures, and their use made our study more comparable to that of Saiag *et al.*,[6] but the psoriatic fibroblasts again had no special effect.

Negative results can never be completely conclusive and it is possible for there to be several additional requirements, such as a need for the epithelial and mesenchymal cells to be in contact. However, the presence of donor dermis in the full thickness skin biopsies employed by Saiag *et al.*[6] meant that such contact was not achieved there. Seeding the keratinocyte suspension directly onto a fibroblast-populated collagen lattice offers an attractive test system, but has been unreliable in our hands and we cannot recall any quantitative proliferation data produced in this way.

The use of baseline counts, as in Table 3, improves the sensitivity of the test system. Such counts were not used in our other experiments because of a scarcity of cells. Even if they had been, the outcome would have been the same: there was no trend in the data which would have been made clearer by subtraction of any postulated level of baseline count.

The length of the experiments is a third consideration. Ours were limited to 2 days to limit confusion of the results from differentiation and detachment of the keratinocytes. This is a sufficient period to detect increased proliferation but a longer test period might be desirable.

A fourth question in the present study was whether the target keratinocytes had been made unresponsive to further stimulation by the presence of epidermal growth factor and other potential mitogens prior to the addition of fibroblasts or their conditioned media. The experiments with leucocyte supernatants served as positive controls, showing that an additional proliferative response was indeed possible. In other studies (Priestley, Lord and Stavropoulos, unpublished) we have also seen a response to minoxidil using the same keratinocyte culture regime.

Epithelial–mesenchymal interactions have important roles in development and in normal homeostasis.[16] Experiments in which various combinations of normal and psoriatic human dermis and epidermis were transplanted separately or together onto nude mice have emphasized the likelihood of such interactions occurring in psoriasis.[16] It is also now clear that cytokines both of epidermal (IL-1) and dermal origin (IL-6, somatomedin C) can influence the behaviour of the underlying or overlying tissues.[17,18] Culture medium from normal keratinocytes stimulates proliferation in fibroblast cultures[19] but the unequivocal demonstration of the opposite effect remains difficult.

In summary, when normal or psoriatic skin fibroblasts, or media conditioned by them, were cultured with normal human keratinocytes for 2 days there was no evidence either that the psoriatic fibroblasts stimulated keratinocyte proliferation or that the normal fibroblasts suppressed it. Our data do not support the hypothesis that the epidermal hyperproliferation in psoriasis is directed by dermal fibroblasts.

ACKNOWLEDGMENT

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Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss

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SUMMARY

Differences in the dynamic skin friction coefficients (μ) were investigated with respect to age, sex, and anatomical region. A total of 29 volunteers consisting of seven young females, seven old females, seven young males, and eight old males participated in the study. Measurements were obtained from 11 anatomical regions, namely, the forehead, upper arm, volar and dorsal forearm, postauricular, palm, abdomen, upper and lower back, thigh, and ankle. The friction data were compared with stratum corneum hydration and transepidermal water loss (TEWL). The dynamic friction coefficient did not vary significantly between age and sex groups but varied considerably among the anatomical regions of the body. The forehead and postauricular had the highest μ ($0{\cdot}34 \pm 0{\cdot}02$) while the abdomen had the lowest ($0{\cdot}12 \pm 0{\cdot}01$); the remaining regions had an average μ value of $0{\cdot}21 \pm 0{\cdot}01$. Similarly, no sex differences were observed for TEWL and stratum corneum hydration. Capacitance was only significantly lower on the palms of the elderly. Regional differences showed a higher state of hydration on the forehead and postauricular as well as the upper arm, upper and lower back when compared with the volar forearm. TEWL was generally lower in the elderly on all anatomical regions except the postauricular and palm. A significant correlation was established between μ and capacitance for most regions. Between μ and TEWL significant correlation was observed only on the palm and thigh. These findings suggest that frictional properties of skin are dependent on more than water content or non-apparent sweating and the role of sebum secretion is suggested as one possible factor.

One basic physical function of the skin is the friction necessary for grasping, gripping, and locomotion. Awareness of the importance of the frictional properties of skin has arisen from

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studies on erosions, abrasions, and blisters which are frequent reactions to trauma, contact-type eczematous reactions and to industrial consequences from poorly-designed work tools, clothing and footwear, and skin care benefits. Several techniques have been described to assess quantitatively the frictional properties of skin.[1–9] Akers and Wolfram reviewed various devices designed to measure skin friction coefficients and to create standardized, reproducible friction damage.[6,10]

Friction is the resistance that opposes movements of one object or medium against another. Amonton in 1699 proposed the law of static friction as: (i) the frictional resistance, F_f, is directly proportional to the load, F_n and (ii) F_f is independent of the area of contact between the surfaces.[5] The ratio between the force necessary to move one of any two surfaces over the other and the load between the two surfaces is thus a constant called the coefficient of friction 'μ' where $F_f = \mu F_n$. The static coefficient of friction was later defined as the force required to start one object in motion past another and the dynamic coefficient as the force required to sustain the motion of one object past another.[5] The latter was found to be usually less than the static coefficient. Subsequent investigations by Comaish and Bottoms, however, found deviations from Amonton's law in skin for a limited range of load forces and attributed this to the viscoelastic deformations of skin.[2] Moreover, other factors such as pressure, stretching, compression, environmental changes of temperature and humidity and individual characteristics such as age, race and sex may be involved in the study of friction.[6]

In this study, we examined age, sex and regional differences in the dynamic skin friction coefficients as well as the latter's association with skin moisture and transepidermal water loss (TEWL).

METHODS

Study population

Twenty-nine volunteers participated having given written consent. The study group consisted of seven young females (aged 24·9 ± 1·1 years, mean ± SD), seven old females (75·3 ± 2·4), seven young males (28·7 ± 0·5), and eight old males (73·8 ± 1·9). All were Caucasians with no signs of skin disease. This study was approved by the University of California San Francisco Committee on Human Research.

Measurements

Measurements were performed on 11 anatomical regions: the forehead (centre), postauricular, upper arm (inner middle third), volar and dorsal forearm (centre between wrist and elbow), palm (thenar aspect), abdomen (an inch above umbilicus), thigh (anterior, upper third), ankle (medial malleoli), upper back (scapula), and lower back (at level of L1).

The dynamic skin friction coefficient, μ, was measured in duplicate using a friction meter (Design Unit, Department of Mechanical Engineering, Newcastle University, Newcastle upon Tyne, U.K.).[3,11] This is a portable hand-held device charged by storage batteries. It consists of a spring-loaded teflon wheel rotating at constant speed and constant contact load so that measurements are done at a specific surface pressure and rubbing velocity and are therefore reproducible. Errors due to the elastic behaviour of skin and to directional surface structure are minimized.[3]

Transepidermal water loss (TEWL) measurements were obtained first to exclude alterations secondary to friction coefficient determinations. An Evaporimeter[12] (Servo Med EP1, Stockholm, Sweden) was used to measure water diffusion from a surface into the air. The probe was held in contact with the skin until a stable TEWL value was established (approximately 30 s). The skin temperature was measured using a thermistor (Tele-Thermometer, Yellow Springs Instruments, Yellow Springs, OH, U.S.A.) taped on to the skin surface. To allow comparison between and within subjects, TEWL values were corrected to a standard skin reference temperature of 30°C.[13] Although uncontrolled in this study, relative humidity (40–57%) and room temperature (18–23°C) were recorded. The fluctuations of relative humidity were too small to detect a correlation considering the variability of measurement.

The electrical capacitance of the skin surface was measured in duplicate using a capacitance meter (Corneometer SM 820 PC, Courage & Khazaka, Cologne, F.R.G.) developed to measure the water content of the stratum corneum.[14,15] This device consists of a main housing and a measuring probe which works as a condensor, the capacitance of which is influenced by a change in the dielectrical constant of the material in contact with the probe. The probe is applied onto the skin with a standard pressure of 3·5 N. Values of actual measurement of moisture appear on the digital display within 3 s after the probe is applied.

Statistics

Differences were tested for their statistical significance using the two-tailed Student's t-test for unpaired data. A P-value less than or equal to 0·05 was considered significant. Correlation coefficients were calculated according to Pearson.[16]

RESULTS

The mean values of dynamic skin friction coefficient, capacitance and TEWL from all volunteers, regardless of age and sex, are shown in Table 1. The dynamic friction coefficient of skin varied considerably among the anatomical regions. The forehead and postauricular had the highest friction coefficients (0·34 ± 0·02) while the abdomen had the lowest (0·12 ± 0·01); the remaining regions had an average μ value of 0·21 ± 0·01. Similarly, measurement of capacitance

TABLE 1. Biophysical skin parameters: regional variability

Region	Dynamic friction coefficient (μ)	Capacitance (i.u.)	TEWL (g/m^2/h)
Forehead	0·34 ± 0·02	94·50 ± 2·58	8·76 ± 0·47
Upper arm	0·23 ± 0·01	88·43 ± 1·85	3·43 ± 0·24
Volar forearm	0·26 ± 0·01	81·66 ± 2·10	3·23 ± 0·26
Dorsal forearm	0·23 ± 0·01	67·05 ± 3·21	3·43 ± 0·22
Postauricular	0·34 ± 0·02	99·76 ± 2·59	5·89 ± 0·43
Palm	0·21 ± 0·01	76·60 ± 4·33	22·64 ± 1·42
Abdomen	0·12 ± 0·01	79·05 ± 2·19	3·35 ± 0·25
Upper back	0·25 ± 0·02	98·81 ± 1·51	4·97 ± 0·41
Lower back	0·19 ± 0·02	95·71 ± 1·59	4·34 ± 0·31
Thigh	0·15 ± 0·01	75·83 ± 2·50	4·15 ± 0·38
Ankle	0·21 ± 0·01	69·41 ± 3·52	7·51 ± 1·12

TEWL, transepidermal water loss; i.u., dimensionless instrument units. Skin friction coefficient μ is dimensionless. Values correspond to mean ± SEM.

as a parameter of stratum corneum hydration showed a higher state of hydration on the forehead and postauricular as well as the upper arm, upper and lower back when compared with the volar forearm. The TEWL was highest on the palm followed by the forehead, ankle, postauricular, upper back, lower back, and thigh.

Table 2 summarizes some of the variations and associations between biophysical parameters within the different anatomical regions. A significant correlation existed (i) between the skin friction coefficient (μ) and capacitance for most regions and (ii) between the friction coefficient and TEWL for the palm and thigh. When μ was plotted against capacitance combining all anatomical regions in the young and old group, respectively, a significant linear correlation was demonstrated (Figs 1a and b).

TABLE 2. Correlation between dynamic coefficient of friction, capacitance and TEWL

Region	Dynamic friction coefficient and capacitance	Dynamic friction coefficient and TEWL
Forehead	0·373*	0·045
Upper arm	0·330*	0·032
Volar forearm	0·460*	0·281
Dorsal forearm	0·455*	0·265
Postauricular	0·161	0·138
Palm	0·570*	0·578*
Abdomen	0·300	0·192
Upper back	0·239	0·148
Lower back	0·330*	0·071
Thigh	0·429*	0·330*
Ankle	0·525*	0·032

Values correspond to correlation coefficient, r. TEWL, transepidermal water loss.

*Significance: $P \leq 0{\cdot}05$, not significant ($P > 0{\cdot}05$) unless indicated.

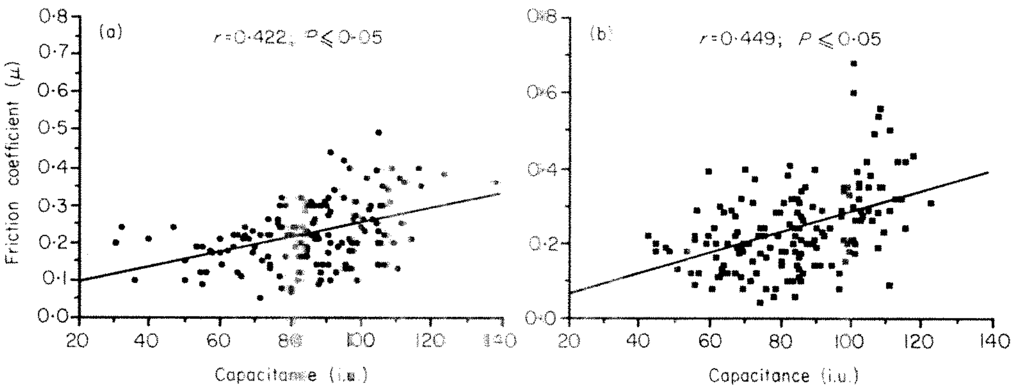

FIGURE 1. Relationship between dynamic skin friction coefficient and capacitance combining all anatomical regions in (a) young and (b) old age groups, respectively. A significant linear correlation was observed between the two parameters for both age groups.

Between age groups, μ did not differ significantly (Table 3). The state of hydration of the stratum corneum also did not differ significantly, except in the palm, where capacitance was significantly lower in the elderly. However, TEWL measurements were significantly lower in the elderly on all body regions except for postauricular and palm.

Comparison between sex groups did not reveal significant differences in μ, capacitance or TEWL as shown in Table 4.

TABLE 3. Friction, capacitance and TEWL: age differences

	Dynamic coefficient of friction (μ)†		Capacitance (i.u.)‡		Transepidermal water loss ($g/m^2/h$)	
Region	Young	Old	Young	Old	Young	Old
Forehead	0·33 ± 0·02	0·35 ± 0·03	94·57 ± 2·24	94·43 ± 4·62	10·19 ± 0·52	7·42 ± 0·59*
Upper arm	0·24 ± 0·02	0·23 ± 0·02	87·04 ± 2·49	89·73 ± 2·75	3·98 ± 0·35	2·91 ± 0·26*
Volar forearm	0·27 ± 0·01	0·26 ± 0·02	81·04 ± 1·66	82·23 ± 3·82	3·91 ± 0·32	2·60 ± 0·33*
Dorsal forearm	0·21 ± 0·01	0·25 ± 0·02	61·64 ± 4·50	72·10 ± 4·31	4·12 ± 0·29	2·77 ± 0·21*
Postauricular	0·31 ± 0·02	0·36 ± 0·03	101·54 ± 2·64	98·10 ± 4·41	5·55 ± 0·29	6·21 ± 0·79
Palm	0·24 ± 0·02	0·19 ± 0·02	87·25 ± 7·85	66·67 ± 2·82*	24·85 ± 2·02	20·72 ± 1·93
Abdomen	0·11 ± 0·01	0·12 ± 0·02	81·68 ± 3·77	76·60 ± 2·33	4·13 ± 0·40	2·62 ± 0·17*
Upper back	0·22 ± 0·02	0·29 ± 0·03	98·96 ± 2·09	98·67 ± 2·25	6·39 ± 0·59	3·75 ± 0·35*
Lower back	0·18 ± 0·02	0·20 ± 0·03	93·89 ± 2·04	97·40 ± 2·40	5·41 ± 0·46	3·41 ± 0·27*
Thigh	0·15 ± 0·01	0·15 ± 0·02	76·04 ± 3·63	75·63 ± 3·57	5·40 ± 0·58	2·98 ± 0·24*
Ankle	0·20 ± 0·02	0·22 ± 0·01	72·21 ± 5·94	66·80 ± 4·03	11·24 ± 1·94	4·28 ± 0·30*

† μ is dimensionless.

‡ i.u., instrument units.

Values correspond to mean ± SEM.

* Statistically significant ($P \leq 0·05$) comparing young vs. old using two-tailed Student's *t*-test.

TABLE 4. Friction, capacitance and TEWL: sex differences

	Dynamic coefficient of friction (μ)*		Capacitance (i.u.)†		Transepidermal water loss ($g/m^2/h$)	
Region	Male	Female	Male	Female	Male	Female
Forehead	0·32 ± 0·02	0·35 ± 0·03	98·13 ± 3·01	90·61 ± 4·11	9·38 ± 0·71	8·09 ± 0·58
Upper arm	0·24 ± 0·01	0·22 ± 0·02	90·30 ± 3·16	86·43 ± 1·75	3·30 ± 0·29	3·56 ± 0·38
Volar forearm	0·28 ± 0·02	0·24 ± 0·02	83·47 ± 3·71	79·71 ± 1·80	3·08 ± 0·39	3·40 ± 0·34
Dorsal forearm	0·23 ± 0·01	0·23 ± 0·02	62·80 ± 5·58	71·61 ± 2·64	3·26 ± 0·26	3·60 ± 0·36
Postauricular	0·35 ± 0·02	0·33 ± 0·03	96·43 ± 4·38	103·32 ± 2·41	6·09 ± 0·79	5·67 ± 0·31
Palm	0·22 ± 0·02	0·20 ± 0·02	73·17 ± 6·28	80·29 ± 6·00	21·90 ± 2·19	23·49 ± 1·80
Abdomen	0·13 ± 0·01	0·10 ± 0·02	80·03 ± 3·56	78·00 ± 2·58	3·10 ± 0·27	3·62 ± 0·43
Upper back	0·27 ± 0·02	0·24 ± 0·03	98·10 ± 2·43	99·57 ± 1·82	4·42 ± 0·45	5·53 ± 0·68
Lower back	0·21 ± 0·02	0·17 ± 0·03	96·97 ± 2·69	94·36 ± 1·65	3·96 ± 0·40	4·72 ± 0·48
Thigh	0·15 ± 0·01	0·15 ± 0·01	76·20 ± 4·26	75·43 ± 2·62	4·21 ± 0·57	4·08 ± 0·51
Ankle	0·21 ± 0·02	0·21 ± 0·01	70·47 ± 5·86	68·29 ± 3·91	7·70 ± 1·50	7·30 ± 1·73

* μ is dimensionless.

† i.u., instrument units.

Values correspond to mean ± SEM.

DISCUSSION

Although the frictional properties of skin have been evaluated with various parameters such as hydration and transepidermal water loss, there has been little emphasis on differences related to age, sex and anatomical region. This study provides a quantitative assessment of differences in the frictional properties of human skin as a function of age, sex and region.

The dynamic friction coefficient did not vary significantly between the young and the old or between males and females. Similarly, no differences were noted between age and sex groups for hydration of the stratum corneum as measured by skin capacitance. Significant differences, however, were observed within regions in both biophysical parameters, and a significant correlation was established between the dynamic skin friction coefficient and capacitance for most regions.

That the skin coefficient of friction changes with the state of hydration has already been established;[8,10,11] extremely dry or wet skin has lower frictional resistance than skin with an intermediate degree of moisture.[1,17] However, this holds only for certain regions such as the forehead, upper arm, volar forearm, thigh and ankle and does not seem to follow for the dorsal forearm or lower back. A relatively lower friction coefficient was found with a higher capacitance in the lower back and vice versa in the dorsal forearm, suggesting that other parameters may be involved in skin friction.

The contribution of TEWL to the frictional properties of the skin is probably not significant since a correlation was observed only in the palm and thigh. Although TEWL was significantly lower in the elderly, the coefficient of friction was not different between age groups.

The role of sweating and sebum secretion has been discussed;[10] similar effects can be obtained with lubricating oils. Petrolatum, a 'greasy' occlusive agent, initially decreases μ due to its lubricating property, but subsequently increases μ over baseline as a result of increased skin moisture due to occlusion.[11] Heavy mineral oil and humectants such as glycerol may hydrate the skin surface by reducing the rate of TEWL, thereby increasing the friction coefficient.[18] It is possible that in spite of higher capacitance in the lower back, the coefficient of friction is lower when compared to the forehead based on a higher sebum content in the latter.

It is evident that the frictional properties of the skin are dependent on more than water content or non-apparent sweating and further investigations are needed to elucidate the complex interactions involved.

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Determination of terbinafine in nail samples during systemic treatment for onychomycoses

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SUMMARY

A combination of alkali treatment and proteinase K digestion was used to solubilize nail samples from patients with onychomycosis treated orally with terbinafine. The samples were extracted and the levels of terbinafine and its metabolite measured by high-pressure liquid chromatography. The results showed a relatively rapid penetration of terbinafine into normal nail plate, suggesting that diffusion via the nail plate is the primary route of entry into this tissue. The levels of terbinafine achieved in nail samples exceeded the range of MICs for dermatophytes at the earliest time point (4 weeks) tested in 50% of patients and remained stable, without accumulation, during periods of treatment of up to 3 months.

Oral antifungal therapy is indicated in onychomycosis because of the deep-seated and persistent nature of this type of infection, and because topically applied drugs do not effectively penetrate the nail plate. Terbinafine is a new allylamine antifungal agent which has recently been shown to be clinically effective in onychomycoses.[1–3] It exerts its antifungal effect by inhibiting the enzyme squalene epoxidase, a key enzyme in ergosterol biosynthesis in fungi.[4,5] The route and speed of penetration of terbinafine into the nail plate is uncertain. The relatively rapid clearance of infected nails suggests that its effectiveness does not rely on incorporation into the slowly growing nail matrix. Determination of the kinetics of terbinafine incorporation into the nail itself is important as this would enable the optimum dose and time of therapy to be determined. At present, systemic therapy for onychomycosis is usually required for 6–12 months. Shortening of the treatment period would increase patient compliance and reduce the risk of side-effects. Since terbinafine is fungicidal for dermatophytes, rapid diffusion throughout the infected nail plate should allow for relatively short treatment times. However, incorporation of the drug into the growing nail would require continuation of therapy until damaged, infected nail had been removed by normal growth. This study was carried out in order to attempt to distinguish between these two possibilities. In addition, the tendency for the drug to accumulate with time was examined. We describe an extraction procedure and a high-pressure liquid

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chromatography (HPLC)-based method for the determination of terbinafine in small (10–20 mg) samples of nail clippings from patients receiving oral therapy for onychomycosis.

METHODS

Chemicals and samples

All solvents were of HPLC grade and all other reagents of Analar grade. All solvents were filtered using a 0·045 μm nylon filter. Proteinase K (Type I fungal proteinase, P0390) was obtained from Sigma Chemical Company Ltd (Poole, Dorset, U.K.). Terbinafine (86-327, Lamisil), demethyl terbinafine (86-621) and internal standard (85-190) were supplied by Sandoz Ltd (Camberley, Surrey, U.K.). Samples of normal human distal nail clippings were obtained from volunteer subjects. At least 40 specimens were obtained from five volunteer subjects for the method validation studies. Samples of normal (uninfected) distal nail clippings were also obtained from 12 patients receiving 250 mg oral terbinafine daily for onychomycosis as part of a multi-centre trial.[2] The informed consent of the patients was obtained prior to samples being taken and the study was given ethical approval by the Joint Ethics Committee of the University Hospital of Wales and University of Wales College of Medicine. All samples were stored for 1–15 months at −20°C until analysed.

Extraction of nail samples

Clippings of distal nail samples, including any attached subungal tissue, were obtained using nail clippers and scissors. Samples of nail (10–20 mg) were weighed and transferred to 15 ml glass tubes. In the method validation studies, standard amounts of terbinafine and demethyl terbinafine were added to the normal nail samples at this stage. To each sample, 1 ml of 1 M NaOH was added, the tubes sealed and the samples left at room temperature for 48 h. The pH was then adjusted to pH 9·0 with 1 M HCl and the molarity adjusted to 0·2 M with distilled water. An aliquot of proteinase K solution (100 μl at 10 mg/ml) was added and the samples incubated at 37°C for 12–16 h. After incubation with proteinase K, the internal standard (50 ng of 85–190) was added and the samples mixed by vortexing. Hexane (3 ml) was added and the samples vortexed for 3 min. To eliminate gel formation, 0·1 ml of methanol was added and the samples centrifuged to assist phase separation (500 *g* for 10 min). After centrifugation, 2·7 ml of the organic layer was transferred to a glass tube and 0·2 ml of 0·5 mol/l H_2SO_4:propanol (85:15) added. The sample was again vortexed for 3 min and after centrifugation, 100 μl of the aqueous phase was injected directly into the HPLC apparatus.

High-pressure liquid chromatography conditions

The apparatus used was a CM4000 solvent delivery system and a SM4000 programmable UV detector (Milton Roy). A Spherisorb C18 column (5 μm, 25 cm) together with a guard column (Apex Prepsil RPC8, 5–20 μm, 2 cm, 2 mm internal diameter) was used for the analysis. Integration was performed using a JCL6000 data aquisition PC system (Jones Chromatography). The eluant was acetonitrile:0·01 M phosphate buffer:triethylamine (80:20:0·1), pH adjusted to 4·0 with 85% phosphoric acid. The flow rate was 2·0 ml/min and the detector wavelength 224 nm. This method is based on that described for the measurement of terbinafine in plasma.[6]

Calculation of recoveries

Comparisons were made between external standards of differing levels of terbinafine and demethyl terbinafine and the calculated values for normal nail samples to which known amounts of these compounds had been added. With clinical samples reference was also made to the internal standard added prior to the extraction procedure.

RESULTS

The HPLC separation of terbinafine and its major metabolite, demethyl terbinafine, in nail samples is shown in Figure 1. Both compounds elute well clear of the bulk of the materials extracted from the nails. The recoveries of varying amounts of terbinafine from spiked nail samples are given in Table 1. Levels of terbinafine could be detected over the range 1–10 ng with

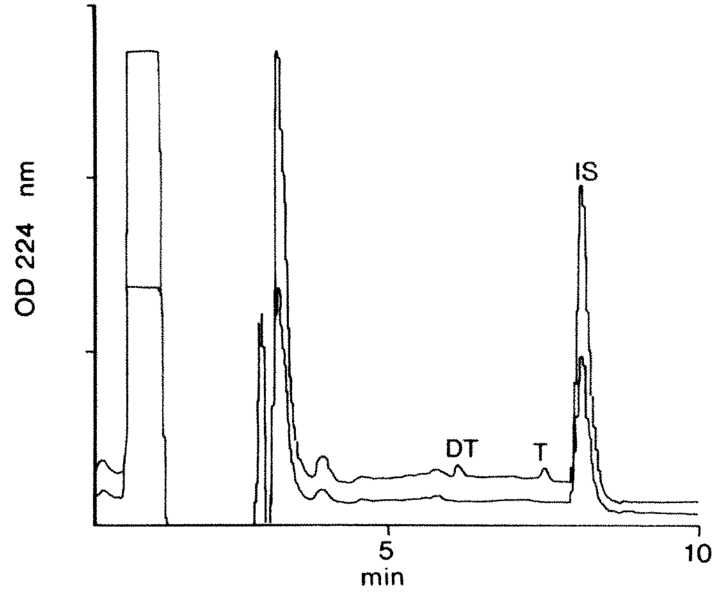

FIGURE 1. HPLC separation of terbinafine and demethyl terbinafine in extracts of human nail. A comparison is made between an extract of nail alone and an extract of nail to which 5 ng of both drug and metabolite have been added prior to extraction. T, terbinafine; DT, demethyl terbinafine; IS, internal standard.

TABLE 1. Recovery of terbinafine after addition of known amounts to normal nail samples

Amount added per 8–12 mg nail	10 ng	5 ng	2 ng	1 ng
Mean recovery, ng ($n = 10$)	9·94	4·58	1·91	0·89
SD	0·84	0·41	0·13	0·12
Coefficient of variation (%)	8·41	9·06	6·88	13·17
Accuracy*(%)	−0·64	−8·51	−4·45	−10·79

$$* \text{ Accuracy} = \frac{\text{Experimental value} - \text{Theoretical value}}{\text{Theoretical value}}.$$

TABLE 2. Recovery of demethyl terbinafine after addition of known amounts to normal nail samples

Amount added per 8–12 mg nail	10 ng	5 ng	2 ng	1 ng
Mean recovery, ng ($n = 10$)	10·17	4·87	2·25	1·08
SD	0·71	0·55	0·21	0·18
Coefficient of variation (%)	6·94	11·37	9·36	16·66
Accuracy* (%)	1·72	−2·68	12·27	8·15

$$* \text{ Accuracy} = \frac{\text{Experimental value} - \text{Theoretical value}}{\text{Theoretical value}}.$$

TABLE 3. Levels of terbinafine and demethyl terbinafine in normal fingernail samples after oral therapy for onychomycosis

	Terbinafine				Demethyl terbinafine			
	Time of sample (weeks)				Time of samples (weeks)			
	0	4	8	12	0	4	8	12
Number detected	0/12	6/12	9/12	11/12	0/12	6/12	9/12	11/12
Mean drug level (ng/mg)	0	0·64	0·28	0·61	0	0·51	0·28	0·37
SD	0	0·56	0·19	0·85	0	0·80	0·22	0·44
Range								
Minimum	0	0·10	0·10	0·10	0	0·17	0·10	0·10
Maximum	0	1·53	0·60	2·89	0	2·14	0·76	1·44

a high level of reproducibility. The recoveries in this range were of the order of 89–99%. As the sample size was in the order of 8–12 mg, this gives a detection limit of approximately 0·1 ng/mg with samples of 10 mg or more. The results with the metabolite, demethyl terbinafine, are given in Table 2. Again a high level of recovery and reproducibility was seen.

The results of drug level measurements in patients receiving oral terbinafine therapy are given in Table 3. In the non-infected nail from the middle finger, terbinafine was detected in six out of 12 patient samples at 4 weeks, rising to 11 out of 12 at 12 weeks. The levels of terbinafine ranged from 0·10–2·89 ng/mg nail, with the average levels being in the range 0·3–0·6 ng/mg (MIC for dermatophytes *in vitro* is 0·001–0·02 µg/ml.[7,8] The levels of terbinafine at 12 weeks did not exceed those at 4 weeks. The levels of the demethyl metabolite of terbinafine showed a similar pattern (Table 3). The results for infected nails did not differ significantly from uninfected samples and the results were reported elsewhere together with the clinical details of this study.[3]

DISCUSSION

Nails are very resistant to solubilization by chemical methods, such as 1 M NaOH or 1M HCl. Even with additional factors such as ultrasonics or heat, the nail samples are not satisfactorily dispersed to ensure optimal solvent extraction. Mechanical disruption by homogenization is not suitable for small samples such as are available in clinical situations. The method described here, a combination of chemical and enzyme digestion, leads to disintegration of the sample together with solubilization. Only occasionally with large samples is there a small residual precipitate. This method therefore forms a satisfactory basis for the measurement of drug levels in human nail samples.

A previous study has described the measurement of an antifungal agent in nails after oral administration.[8,9] In this study the levels of itraconazole were measured in nail samples from two normal volunteers receiving 100 mg itraconazole daily. Nail samples were ground and then homogenized before extraction.[10] This approach has not proved satisfactory in our study, particularly with the small (10–20 mg) clinical samples. The method described here is suitable for the small samples from individual nails and thus allows monitoring of infected as well as normal nail samples from the same individual during oral therapy.

Theoretically, the incorporation of oral antifungal agents into nail samples may be by one of two methods. Firstly, the drug may be incorporated into the growing nail at the matrix. A band of drug would then move along with the nail as the nail grows, clearing the infection.[11] Alternatively, the drug could diffuse into the nail from the nail bed. If this were the case, fungicidal levels would be reached in the distal nail samples at an early stage after therapy. The results presented here clearly show that orally administered terbinafine is present at fungicidal levels in distal nail clippings as early as 4 weeks after commencing treatment. It would therefore seem that with terbinafine, diffusion through the nail plate is the primary route of penetration. The lack of increase of terbinafine and its metabolite with time would suggest that there is no long-term accumulation in nail and that the nail compartment rapidly reaches an equilibrium with other body compartments.

ACKNOWLEDGMENTS

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Epidermal interleukin 1 in normal skin of patients with HIV infection

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SUMMARY

Interleukin 1 (IL-1) in the epidermis plays an important role together with Langerhans cells in the activation of the T cells. To determine whether IL-1 could be located in the normal epidermis of 54 patients with HIV infection and related to the stage of the disease, an indirect immunofluorescence technique was used. Intense epidermal fluorescence to IL-1 was noted in the asymptomatic stage II of the disease, whereas there was minimal reactivity in the other stages.

Interleukin 1 (IL-1) is an important cytokine that amplifies T-cell function by inducing the production of interleukin 2 (IL-2) and the expression of the IL-2 receptor. It also plays an important role in both local and systemic inflammatory reactions.[1] IL-1 has been found in normal, unstimulated human epidermis,[2,3] although originally it was described in monocytes. IL-1 and the Langerhans cells play an essential role in the activation of T lymphocytes in the skin and the initiation of a local inflammatory reaction.

Patients infected with the human immunodeficiency virus (HIV-1) develop many opportunistic cutaneous infections. It has been shown that the Langerhans cells were decreased in the normal epidermis of patients with HIV infection,[4,5] especially in those with the more advanced stages of the disease.[6] So far, there have been no studies on the role of epidermal IL-1 in the development of these cutaneous infections. In this study IL-1 was investigated in the skin to determine its localization in the epidermis of patients with HIV infection at different stages.

METHODS

Patients

Fifty-four patients with serologically proven HIV-1 infection (35 men and 19 women, age range 18–45 years) who had received no immunosuppressive drugs, topical corticosteroids, radiotherapy or photochemotherapy in the previous 2 months were included in this study. These

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TABLE 1. Centre for Disease Control (CDC) classification of disease stages in HIV infection

Stage	Symptoms
I	Acute infection
II	Asymptomatic infection
III	Persistent generalized lymphadenopathy
IVa	Systemic features
IVb	Neurological disease
IVc	Secondary infections
IVd	Secondary cancers: Kaposi's sarcoma and lymphoma
IVe	Other conditions

patients were classified according to the CDC classification as shown in Table 1. Twenty-one patients were asymptomatic (stage II) and 14 had persistent generalized lymphadenopathy (stage III). Of the patients 12 had secondary infections (stage IVc) and seven had various malignancies (stage IVd). Controls consisted of 15 healthy heterosexuals who were not in the risk groups and who had negative serology with no cutaneous lesions.

Skin biopsies

Punch biopsies (4 mm) were obtained from normal skin on the sun-protected side of the upper arm of the patients positive for HIV and from normal controls. These skin biopsies were snap frozen in liquid nitrogen and stored at −70°C.

A rabbit polyclonal anti-human IL-1 was obtained from Genzyme Co (Boston, MA, U.S.A.). This antibody binds to human pI 5·0 and pI 7·0 IL-1 from both natural and recombinant sources, and also to the IL-1 precursor 31 kDa molecule.[7] The antibody is species specific and does not cross-react with other lymphokines. Sections (4 μm) were cut and immersed for 10 min in acetone and then washed with phosphate-buffered saline (PBS). The tissue sections were then incubated with anti-human IL-1 diluted 1/200 for 20 min at 37°C. The sections were washed in PBS and incubated with a fluorescein-conjugated goat anti-rabbit IgG, diluted 1/20, for 30 min at 37°C and then washed in PBS. The sections were mounted in buffered glycerin and examined using a Leitz fluorescence microscope.

Negative controls were performed on normal skin from HIV patients and from the controls. The specific staining was lost if either the primary antibody (anti-IL-1) or the second labelled antibody (fluorescein-conjugated goat anti-rabbit IgG) was omitted. Also, the specific staining disappeared if the anti-IL-1 was replaced by an irrelevant IgG polyclonal antibody.

The intensity of the labelling in the epidermis was graded as follows: −, negative; +, weak; + +, moderate; + + +, intense. A mean was established by examining four fields at ×40 magnification of three separate sections from each patient.

Blocking studies

To test the specificity of the labelling with the IL-1 antibody, this was pre-incubated with purified human IL-1 (200 U/ml) at different dilutions for 30 min at room temperature before staining. Another cytokine, TNFα (400 U/ml) was used as the irrelevant control.

TABLE 2. Interleukin 1 reactivity of normal epidermis in 54 HIV patients at different stages of affection

	IL-1 Epidermis			
Stage	−	+	+ +	+ + +
II	0	0	3	18
III	0	3	9	2
IVc	7	5	0	0
IVd	0	1	6	0

−, negative; +, weak; + +, moderate; + + +, strong.

RESULTS

The results are summarized in Table 2.

Fluorescent staining of epidermis with anti-IL-1

Of the stage II patients, 18 showed strong (+ + +) labelling with either intercellular (15 patients) as shown in Figure 1, or cytoplasmic fluorescence (three patients), as shown in Figure 2. Three patients had moderate intercellular staining. The intercellular reactivity was similar to

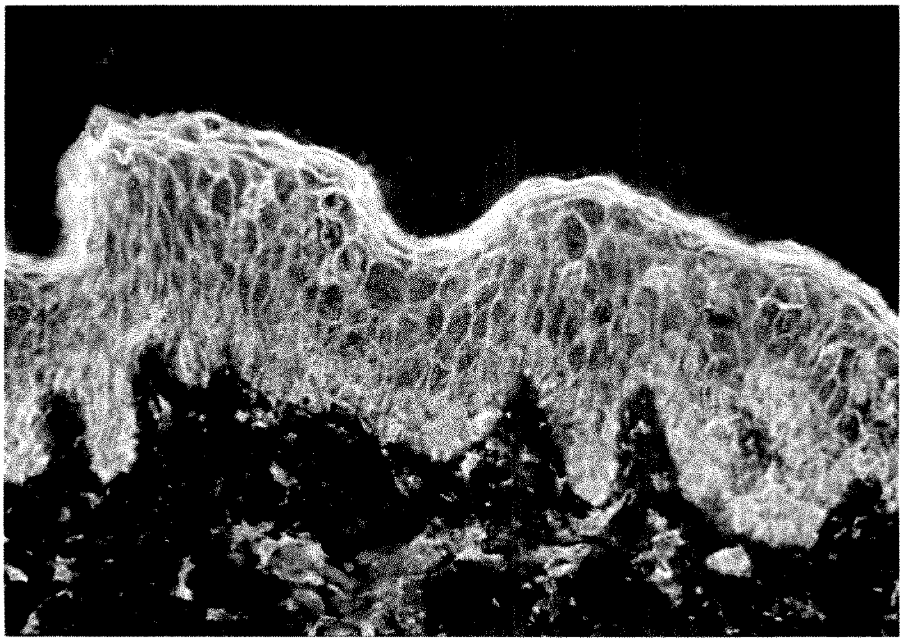

FIGURE 1. Strong intercellular labelling with IL-1 antibody at stage II (× 300).

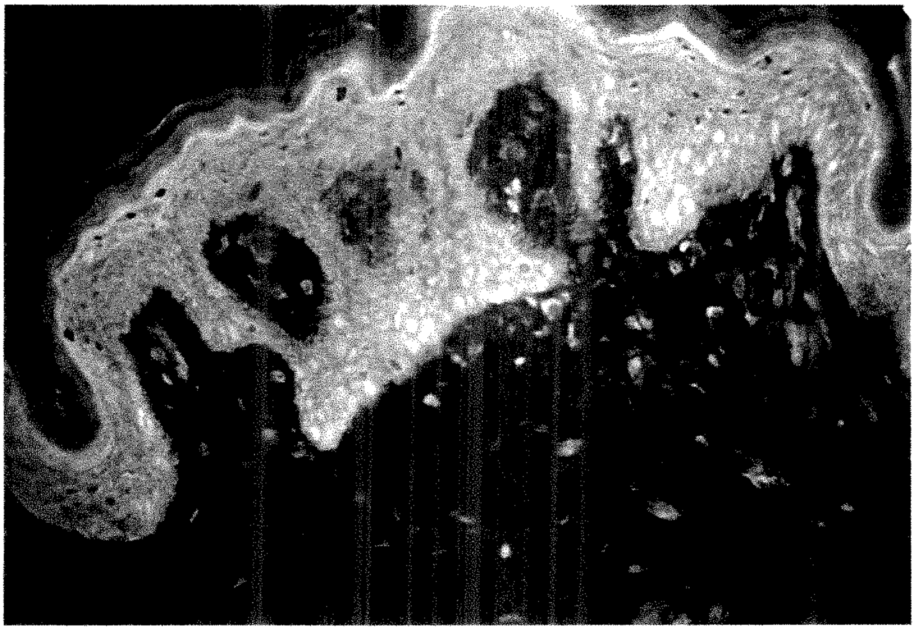

FIGURE 2. Strong cytoplasmic labelling with IL-2 antibody at stage II (× 300).

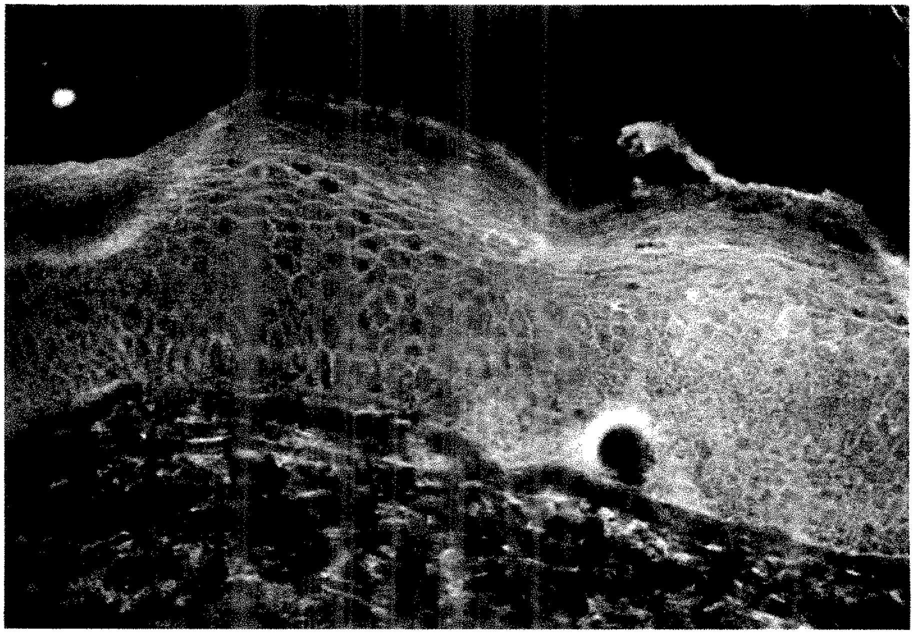

FIGURE 3. Weak intercellular labelling with IL-1 antibody at stage IVc.

that observed on immunofluorescence in pemphigus vulgaris, and involved the full thickness of the epidermis. Of the stage III HIV patients, the labelling was weak (+) in three patients, moderate (+ +) in nine patients and was strong (+ + +) in three. In stage IVd patients the labelling was weak (+) in one of the patients and moderate (+ +) in six patients. The labelling was negative in seven patients with stage IVc and weak in five (Fig. 3). The labelling was essentially intercellular and was localized at the granular layer.

Of the 15 normal controls, nine had a weak (+) intercellular labelling on the granular layer and six were negative.

Blocking study

Pre-incubation of IL-1 antibody with human IL-1 inhibited the labelling in a dose-dependent fashion. The complete inhibition of the labelling was obtained at a 1/2 dilution. Pre-incubation with TNFα did not inhibit the labelling.

DISCUSSION

This study revealed an intense epidermal reactivity in the normal epidermis of stage II patients infected with HIV but with no cutaneous lesions. However, at later stages of the disease the epidermal labelling was weak and was negative in seven patients in stage IVc, although these had opportunistic infections and sometimes cutaneous lesions. The epidermal reactivity was inhibited by pre-incubation of IL-1 antibody with IL-1, which demonstrated its specificity. A previous study of cutaneous T-cell lymphomas[8] demonstrated the advantage of an *in situ* direct immunofluorescent technique for the identification of IL-1 in the epidermis, especially for preservation of antigenic determinants and the localization of IL-1 at specific sites.

An increase in the spontaneous production of IL-1 in the plasma by monocytes from patients with AIDS-related complex has been previously demonstrated[9] as compared with the decreased IL-1 production by activated monocytes in those patients with AIDS. This suggests that in the early stages of HIV infection adherent cells were activated and became deficient with the progression of the disease. This study indicates a similar phenomenon in the skin and that HIV induces an immunological activation with the production of IL-1 in the epidermis of HIV patients at an early stage of the disease and when they have no cutaneous lesions. This activation occurs even earlier than in plasma which occurs at stage III. This could be directly related to the infection of Langerhans cells by the virus.[10] However, IL-1 skin reactivity was weak or negative in stages III, IVd and IVc, in association with a decrease in the number of Langerhans cells in the normal epidermis.[6] Since IL-1 and Langerhans cells induce the immunological reaction, their deficit in the normal epidermis of HIV patients in stages III and IV could result in abnormalities in T4 cell activation and the inflammatory reaction in the skin of these patients. Thus, there could be a promotion of the development of cutaneous infections. Moreover, IL-1 plays an important role in the maturation of the epidermal Langerhans cells.[11]

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Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis

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SUMMARY

The aerobic bacterial flora of 20 patients with atopic dermatitis and 19 control subjects was extensively sampled using the Williamson and Kligman scrub technique and a contact-plate method (cysteine lactose electrolyte deficient media). Comparison of the two quantitative techniques showed that the contact plate is a reliable and convenient alternative to the scrub technique for the quantification of *Staphylococcus aureus*, micrococci and coagulase negative staphylococci. Quantification of bacterial flora using both techniques showed high rates of colonization by *S. aureus* on both involved and clinically normal skin of patients with atopic dermatitis. A linear increase in *S. aureus* counts with increasing severity of dermatitis was found. In contrast, diphtheroids showed a trend of decreasing isolation rates and counts as the severity of the dermatitis increased. Isolation rates and absolute counts for micrococci/coagulase negative cocci were unaffected by the severity of the dermatitis.

Profound changes in cutaneous flora occur in patients with atopic dermatitis.[1–4] Both dermatitic and clinically normal skin is colonized by *Staphylococcus aureus* in contrast to normal subjects where *S. aureus* rarely forms part of the normal flora. The presence of *S. aureus* may increase the activity of atopic dermatitis[5] and can serve as a source of cross-infection.[4] The increased colonization by *S. aureus* in patients with atopic dermatitis may be caused by enhanced adherence of *S. aureus* to skin and mucous membranes[6–9] due to the presence of receptors on the cell wall of *S. aureus* for fibronectin[10] and laminin[11] present in the tissues. Assessments of micrococci, coagulase negative staphylococci (coag. neg. staphs) and diphtheroids on the skin of patients with atopic dermatitis have yielded variable results.[2,12]

Most quantitative studies of bacterial flora have used the scrub technique of Williamson and Kligman[13] and in atopic dermatitis have been restricted to two areas of the body, one with active atopic dermatitis and the other with clinically normal skin.[1–4] Human cutaneous microflora

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varies greatly with body site,[14] but regional variation in flora has not been studied before in atopic dermatitis. Few reports have been published on the correlation of the density of the Staphylococcal colonization with the severity of atopic dermatitis although a reduction in *S. aureus* counts has been found as dermatitis treated with topical corticosteroids improves.[15] Leyden *et al.* suggested a figure of 10^6 colony forming units (c.f.u.) per cm^2 as being indicative of clinical infection requiring specific antibacterial therapy.[3] The paucity of studies of extensive bacterial sampling in patients with atopic dermatitis may be because such a study would be very time consuming both clinically and in the laboratory if the scrub technique were used. Faster direct and indirect impression methods of bacteriological sampling have been suggested.[16] A direct contact plate formed from cysteine lactose electrolyte deficient agar (CLED) (Oxoid Ltd., U.K.) has recently been used in a study of atopic dermatitis.[17] If reliable, the use of such a contact plate would allow widespread sampling to be undertaken quickly. It would also be applicable to young children, the largest group of atopic dermatitis patients. Multiple sampling with the scrub technique in children is impractical because a subject must remain completely still for periods of 2–3 min while each site is sampled.

This study had two main aims: to compare the CLED contact plate and the scrub technique for the isolation and quantification of cutaneous aerobic bacteria and to investigate the cutaneous flora of patients with atopic dermatitis in relation to both clinical disease severity and changes across time and body site.

METHODS

Clinical details

Twenty patients with atopic dermatitis fulfilling the criteria of Hanifin and Rajka[18] and 19 control subjects with no history of skin disease took part in the study. The two groups were comparable for age and sex (atopics 11 males, 9 females, median age 29 years; controls 12 males, 7 females, median age 27 years). Ethical committee approval was obtained and all subjects gave informed consent. Any individual who had received either systemic or topical antibiotics in the preceding 4 weeks was excluded from the study. All participants were asked not to wash or apply any topical treatment during the 6 h prior to sampling.

Bacteriological details

Methods of sampling. Quantitative bacterial examination was undertaken at 19 standard body sites using two different methods (Fig. 1). These consisted of (a) the scrub technique of Williamson and Kligman[13] and (b) a contact plate of surface area 24 cm^2 (Sterilin U.K.) filled with CLED agar.[17] Qualitative investigation was undertaken at the four additional sites shown in Figure 1 using rayon-tipped swabs (Albuswabs, Exogen, Clydebank, U.K.). At each sampling site the skin was assessed by scoring any dermatitis on a 1–3 (mild to severe) scale and coding clinically normal skin 0. When two techniques were used at one site, directly adjacent areas of skin of similar clinical dermatitic activity were sampled.

Laboratory.

(a) Scrub technique of Williamson and Kligman. Tenfold dilutions of the scrub fluid were plated onto blood agar (nutrient agar, Oxoid U.K., Ltd, plus 7·5% defibrinated horse blood) and incubated aerobically at 37°C for 24 h. Colony forming units per cm^2 of skin sampled were then calculated by standard methods and expressed as $\log_{10}$ values.

(b) Contact-plate method. The CLED contact plate was incubated aerobically at 37°C for

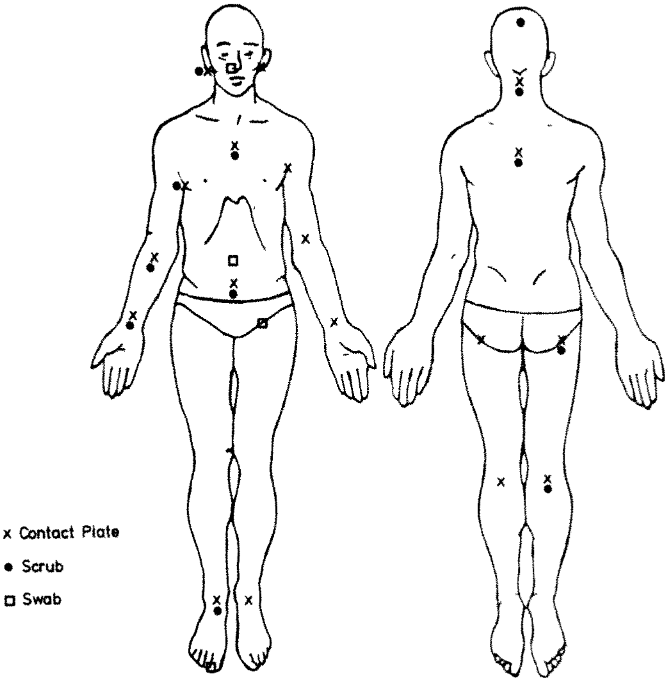

FIGURE 1. Sites and techniques of bacterial sampling.

24 h. Colony forming units per 24 cm^2 were counted directly from the plate and again expressed as $\log_{10}$ values. For statistical analysis confluent growth was assigned the numerical estimate of 1800 c.f.u. per 24 cm^2 and semiconfluent growth 1400 c.f.u.

(c) Swabs. Swabs were plated onto blood agar and incubated aerobically at 37°C for 24 h. Bacteria isolated were then recorded.

Bacterial identification. Bacteria were identified using standard laboratory techniques and differentiated into *S. aureus*, micrococci/coag. neg. staphs. and diphtheroids. The differentiation between *S. aureus* and other micrococci/coag. neg. staphs. was based on the result of a slide latex agglutination test (Staphaurex Test, Wellcome U.K.). All isolates of *S. aureus* were phage typed using the International basic set of *S. aureus* typing phages.

Statistical methods

Bacterial counts. Throughout the analysis it was assumed that the logarithm of the bacterial count followed a normal distribution. This was validated by probability plots and plots of residuals as appropriate.

Comparison of scrub and contact-plate techniques. Qualitatively, a simple comparison of bacterial isolation achieved by the two techniques was made between paired samples. Quantitatively, two measures of correlation between the counts obtained by the two sampling techniques were calculated. Firstly, within-person correlations were evaluated and summary statistics across the sample are provided. Secondly, as a measure of between-person correlation the mean count per site was calculated for the two techniques and then correlated across the full sample. As the assumption of normality for the logged counts was appropriate (both univariate and bivariate) the usual product-moment estimate of correlation was used throughout.

Effect of dermatitis. Separate mean log counts for the dermatitic and non-dermatitic sites within the atopic group and the control group were calculated for each of the two quantitative techniques. Confidence intervals (95%) for the differences between the above means were calculated according to the method of Gardner and Altman.[19] To assess whether the severity of dermatitis affected the bacterial count when individual patient and body site effects had been accounted for, a general linear model was fitted to incorporate all three possible effects using the package GLIM (Royal Statistical Society, London). This model was also used to quantify the relative importance of the effect of body site and between individual variation on bacterial counts compared to the effect of dermatitis activity in terms of the ratios of mean squares due to the effects.

RESULTS

Comparison of scrub and contact-plate sampling techniques

Qualitatively, the two techniques agreed on the presence or absence of the respective bacterium in the majority of observations (range of agreement 59–90%, Table 1). This applied to all three bacterial classifications in both the atopic and control groups. Where disagreement existed it was far more common for the contact-plate method to isolate the organism in the presence of a negative result by the scrub technique than vice versa. Quantitatively, log c.f.u. 24 cm^{-2} for the contact plate were smaller by a factor of approximately 2 compared with log c.f.u. cm^{-2} for the scrub technique. Table 2 gives a summary of all the correlations within sites for each subject as well as between subjects for each group (the latter based on the average log count across sites for that subject). The vast majority of these correlations are positive and significantly greater than zero. For the atopic group *S.aureus* and micrococci/coag.neg. staphs. both show a reasonable but inexact relationship between the two techniques (median within subject correlations of 0·59 and 0·49, respectively, and between subject correlations of 0·41 and 0·55).

Bacterial counts

Patterns of isolation and bacterial counts differed for the three groups of organisms.

TABLE 1. Qualitative comparison of contact plate and scrub techniques (Percentage of total paired samplings showing agreement or disagreement on bacterial isolation)

	Agreement		Plate pos., Scrub neg.		Plate neg., Scrub pos.	
	Atopic (%)	Control (%)	Atopic (%)	Control (%)	Atopic (%)	Control (%)
S. aureus	75	90	20	8	5	3
Micrococci/ coag. neg. staphs.	59	72	36	27	5	2
Diphtheroids	63	65	30	31	7	4

TABLE 2. Correlation between sampling techniques. *r* values for correlations between positive results (see text for method of calculation)

	Atopic group		Control group	
	Within* subject	Between subject	Within* subject	Between subject
S. aureus	0·59 (0·41→0·74)	0·41	†	†
Micrococci/coag. neg. staphs.	0·49 (0·32→0·83)	0·55	0·51 (0·29→0·77)	0·55
Diphtheroids	0·09 (−0·30→0·52)	−0·55	0·48 (0·18→0·63)	−0·19

* Median values plus interquartile ranges.
† Insufficient isolates for statistical analysis.

S. aureus. Using the contact-plate method, atopic patients showed increased *S. aureus* isolation rates on both dermatitic (91%) and clinically normal skin (49%) compared to control group individuals (11%) (Tables 3 and 4). Bacterial counts showed a similar statistically significant trend (log mean counts dermatitic skin 2·19, clinically normal skin 1·26 and controls 0·68). Isolation rates were lower using the scrub technique (78, 32 and 14%, respectively), though as expected the counts were higher (4·55, 3·84 and 1·62). Patients with atopic dermatitis also showed a progressive increase of *S. aureus* counts with increasing severity of dermatitis ($P < 0·01$) and an increase in the proportion of the total bacterial count made up by *S. aureus* (Figs 2 and 3). The effect of dermatitic activity on *S. aureus* counts was very much greater than that seen with between body site variation and also greater than the effect of between individual variation (Table 5). All the above findings were confirmed using both quantitative sampling techniques.

Phage typing of isolates from the atopic group showed that the largest proportion were members of group 3 (group 1, 3%, group 2, 19%, group 3, 46%, others 16% and non-typable 16%). In many individuals single sites carried more than one phage type and 50% of patients carried more than a single phage type group at any one time.

Six individuals from the atopic group were resampled on five further occasions over a 30-week period. *S. aureus* counts for each site within each individual varied greatly across time but at all times showed a strong relationship with dermatitic activity ($P < 0·05$). In these six individuals a single phage type generally predominated throughout the 30-week study period but other phage types were frequently isolated on one or two sampling occasions.

Micrococci/coag. neg. staphs. In contrast to *S. aureus*, isolation rates for micrococci/coag. neg. staphs were similar for the atopic affected and unaffected skin and control groups (Tables 3 and 4). Bacterial counts however, were significantly lower in controls as compared with atopic patients ($P < 0·05$). No differences were found between clinically normal skin in atopic patients as compared with affected skin (Table 3). As dermatitis activity increased, micrococci/coag. neg. staphs counts remained static (Fig. 2) though they formed a smaller proportion of the increasing

TABLE 3. Summary of bacterial counts

	Contact plate				Scrub			
	Number of Sites	Isolation rate (%)	Mean of log counts*	95% CI dif	Number of sites	Isolation rate (%)	Mean of log counts*	95% CI dif
S. aureus								
Atopic dermatitic sites	182	91	2·19	0·74, 1·12†	114	78	4·55	0·34, 1·08†
Atopic non-dermatitic sites	120	49	1·25	0·43, 0·73†	84	32	3·84	1·99, 2·45†
Controls (normal skin)	341	11	0·68		216	14	1·62	
Micrococci/coag. neg. staphs.								
Atopic dermatitic sites	182	82	1·85	−0·31, 0·07	108	59	4·54	0·24, 0·86†
Atopic non-dermatitic sites	120	83	1·97	0·43, 0·73†	79	62	3·99	0·76, 1·46†
Controls (normal skin)	340	88	1·39		226	61	2·88	
Diphtheroids								
Atopic dermatitic sites	182	68	1·37	−0·54, −0·16†	114	40	4·38	−0·09, 0·89
Atopic non-dermatitic sites	120	82	1·72	0·16, 0·45†	84	55	3·98	0·78, 1·50†
Controls (normal skin)	337	87	1·41		227	69	2·84	

95% CI dif 95% confidence intervals of differences between means.
*Positive counts only.
†Significant differences ($P < 0·05$).

TABLE 4. Swab investigations: Percentage isolation

	S. aureus		Micrococci/coag. neg. staphs.		Diphtheroids	
Site	Atopic (%)	Control (%)	Atopic (%)	Control (%)	Atopic (%)	Control (%)
Nares	90	53	85	84	45	84
Umbilicus	60	5	80	84	55	84
Groin	60	5	80	68	45	84
Toe clefts	45	5	90	79	45	84

total bacterial count (Fig. 3). Unlike *S. aureus*, body site and between individual variation had a much greater effect on the bacterial counts than dermatitic activity (Table 5).

Diphtheroids. Diphtheroids were isolated more commonly from the clinically normal skin in patients with atopic dermatitis and control subjects than from the dermatitic areas of atopic dermatitis patients (Tables 3 and 4). However, despite similar isolation rates, significantly lower

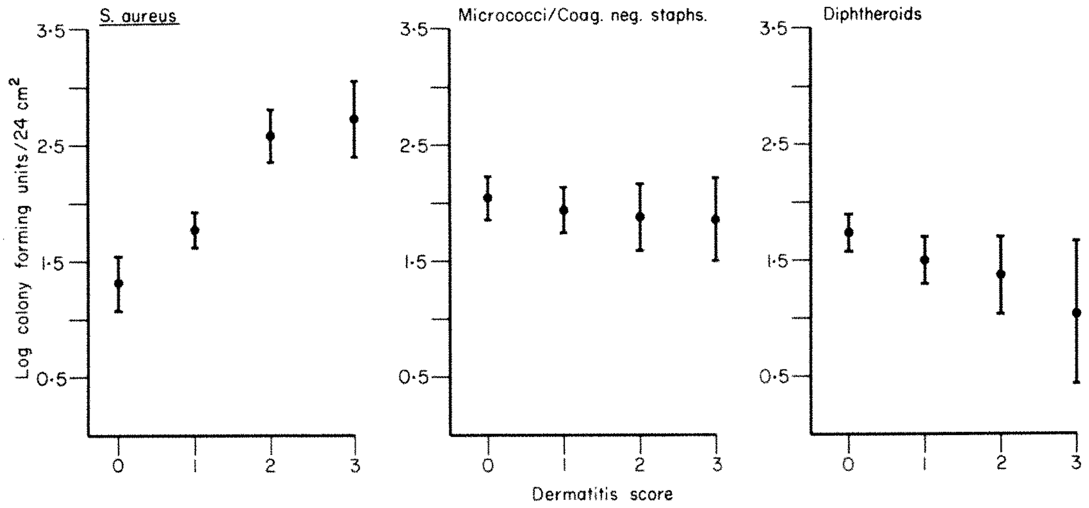

FIGURE 2. Variation of bacterial counts with severity of dermatitis (contact-plate method). Means with 95% confidence intervals. Atopic group.

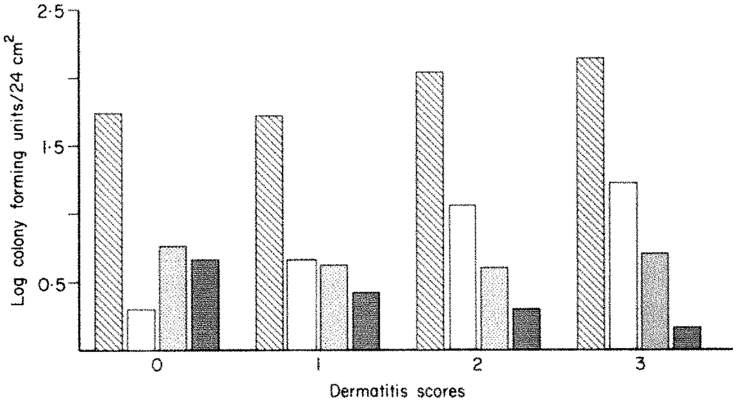

FIGURE 3. Mean bacterial counts for each dermatitis score. Total counts and individual bacterial group counts expressed as a proportion of the total count. (contact-plate method). Atopic group. ▧, total counts; □, *S. aureus*; ▩, micrococci/coag. neg. staphs; ■, diphtheroids.

counts were seen in the control subjects compared with the unaffected skin of atopic patients. A trend for diphtheroid counts to decrease with increasing dermatitis severity was noted (Fig. 2) but this did not reach statistical significance. The effect of dermatitis activity on bacterial counts was found to be less than that of body site and much less than between individual variation.

DISCUSSION

The contact-plate method is fast and convenient to use clinically and the plates can be directly incubated in the laboratory. Qualitatively, this study has found it to be more sensitive than the

TABLE 5. Relative effect of dermatitis activity, subject and body site on bacterial counts (Ratio of mean squares, linear model, GLIM package.) (Atopic group.) This model assigns the arbitrary value of 1 to the variation in bacterial counts ascribeable to dermatitis severity and measures the effects of body site and between person variation on the bacterial counts relative to that

	Dermatitis activity	Subject	Body site
S. aureus	1	0·53	0·07
Micrococci/coag. neg. staphs	1	51·00	32·00
Diphtheroids	1	9·00	1·80

scrub technique, probably because of the larger area of sampling. Quantitatively, the results correlate well with those obtained by the scrub technique for *S. aureus* and micrococci/coag. neg. staphs. although it appears to be less satisfactory for the diphtheroid group of organisms. The contact-plate method does however, give lower absolute counts than the scrub technique. There are two possible explanations for this. Firstly the contact-plate samples only the most superficial layer of the skin and secondly, only a small number of the members of each colony are sampled by the contact plate rather than most members as in the scrub technique.[13] Lower absolute numbers may be a disadvantage where a more sensitive indication of change in colony counts is required. However, the contact plate allows multiple sampling in a single patient because of increased speed of the technique. The scrub technique is therefore indicated for studies where small areas of skin are involved or where relatively small quantitative changes in flora are anticipated. The contact plate provides a reliable and fast alternative in paediatric studies and where more extensive bacterial sampling is desired.

Isolation rates and counts of *S. aureus* in atopic dermatitis in this study are similar to those previously reported[1-4] but provide a more comprehensive anatomical survey of the body surface. The close association of dermatitis and *S. aureus* has been confirmed by the overwhelming influence of dermatitis activity on *S. aureus* counts compared to that of body site or between person variation. The linear increase in *S. aureus* counts with increasing dermatitis activity (Fig. 2) suggests that there is no clear quantitative boundary between colonization and clinical infection as has been suggested by Leyden *et al.*[3] Antibacterial therapy at any level of atopic dermatitis severity may be clinically beneficial.[17] However, long-term antibiotic treatment, topical or systemic, cannot be recommended for patients with atopic dermatitis because of the risk of developing bacterial resistance. Alternative methods of reducing bacterial numbers such as antiseptics might be suitable.

Phage types in the atopic group were similar to those reported previously by us.[17] Our results for the six patients followed over a 30 week period suggest that the *S. aureus* strain is relatively stable for any individual over time with the addition of other transient strains.

The relatively small effect of dermatitis on isolation rates and counts for micrococci/coag. neg. staphs shown in this study confirms previous reports. Strict comparison with those studies is difficult as we did not differentiate between micrococci and coag. neg. staphs.[1,12] The lack of any special association between coag. neg. staphs. and atopic dermatitis is also supported by *in vitro* adherence studies.[8]

The lower isolation rates and bacterial counts for the diphtheroid group of organisms shown in this study has also been previously reported in patients with atopic dermatitis.[1] This could be explained by differences in adherence of diphtheroids to keratinocytes in patients with atopic dermatitis although this has not yet been studied. Alternatively the diphtheroids could simply be overwhelmed by the increasing numbers of *S. aureus*.

In summary, the bacteriological environment in atopic dermatitis shows increasing numbers of *S. aureus* with increasing severity of dermatitis, a trend for diphtheroids to decrease with increasing dermatitis severity and relatively stable numbers of micrococci/coag. neg. staphs. Studies of the effect of antibacterial therapy on atopic dermatitis can now include extensive bacterial sampling both in children and within a busy clinic using the CLED contact-plate technique which has proved a reliable qualitative and quantitative assessment method.

ACKNOWLEDGMENT

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Effect of incisional biopsy on subsequent histology of melanocytic naevi

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SUMMARY

We examined the effect of incisional biopsy on the subsequent histology of 15 benign melanocytic naevi. In all cases the histology of the residual lesion within the second specimen was unchanged apart from the presence of a surgical scar, and in general the definitive excision specimen showed a reduction in melanocytes at the dermo-epidermal junction overlying the scar. In no case were there any features suggestive of melanoma.

We have long held the view that the majority of dysplastic naevi mature into banal, predominantly intradermal naevi with a loss of the atypical junctional component. To test this hypothesis[1] would require serial biopsies of individual lesions and such a study would only be possible if the original incisional biopsy did not affect the subsequent histology. However partial removal of benign melanocytic naevi by techniques such as shave excision is reported to produce histological changes in the scar resembling those seen in melanocytic dysplasia or even superficial melanoma. [2–3] To see whether such changes also follow incisional biopsy we have examined the effect of incisional biopsy upon the subsequent histology of 15 melanocytic naevi as a preliminary to a study of the maturation of naevi by serial biopsies.

METHODS

A total of 15 lesions were studied. Six naevi were biopsied using a 3-mm punch either 6 or 15 weeks prior to definitive excision. Nine large (> 1 cm) naevi at anatomical sites where excision and primary closure would have been difficult were removed in two stages, the second after an interval of 3–26 weeks. Multiple blocks of all specimens were fixed in 10% phosphate-buffered formalin, processed and embedded in paraffin wax. Representative 5 μm sections were cut and stained with haematoxylin and eosin. The first biopsies were categorized with regard to naevus type, and the subsequent excision specimens were closely compared with the original biopsy and

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TABLE 1. Details of biopsies

Case no.	Histology	Type of biopsy	Weeks between biopsies
1	Intradermal naevus	Ellipse	10
2	Compound naevus with architectural melanocytic dysplasia	Ellipse	9
3	Compound naevus	Ellipse	8
4	Intradermal naevus with congenital pattern	Ellipse	9
5	Compound naevus with melanocytic dysplasia	Ellipse	23
6	Compound naevus with melanocytic dysplasia	Punch	6
7	Compound naevus	Punch	6
8	Compound naevus (congenital) with mild melanocytic dysplasia	Punch	6
9	Junctional naevus	Punch	6
10	Compound naevus	Punch	15
11	Compound naevus	Punch	15
12	Junctional naevus with melanocytic dysplasia	Ellipse	3
13	Compound naevus with congenital pattern	Ellipse	26
14	Junctional naevus with melanocytic dysplasia	Ellipse	4
15	Compound naevus	Ellipse	25

examined in particular for melanocytic atypia, increased junctional activity and repopulation of the epidermis adjacent to the scar by melanocytes.

RESULTS

Details of the biopsies are shown in Table 1. Histologically the 15 naevi were: compound naevus with mild to moderate melanocytic dysplasia (4), junctional naevus with mild to moderate melanocytic dysplasia (2), benign intradermal naevus (1), intradermal naevus with a congenital pattern (1), compound naevus (5), compound naevus with a congenital pattern (1) and junctional naevus (1). In all cases the histology of the residual lesion within the second specimen was identical to the first with the addition of focal scarring. In 13 of the second specimens excised 3–26 weeks after incisional biopsy, the dermo-epidermal junction was flat with loss of rete ridges and with very few melanocytes in the epidermis overlying the scar (Fig. 1); while in the remaining two lesions (excised 6 and 25 weeks after incisional biopsy) the number of melanocytes was equal to or slightly greater than in the original biopsy (Fig. 2). In one case there was some melanocytic atypia but no more than is commonly seen in benign junctional melanocytic lesions. None showed features suggestive of melanoma.

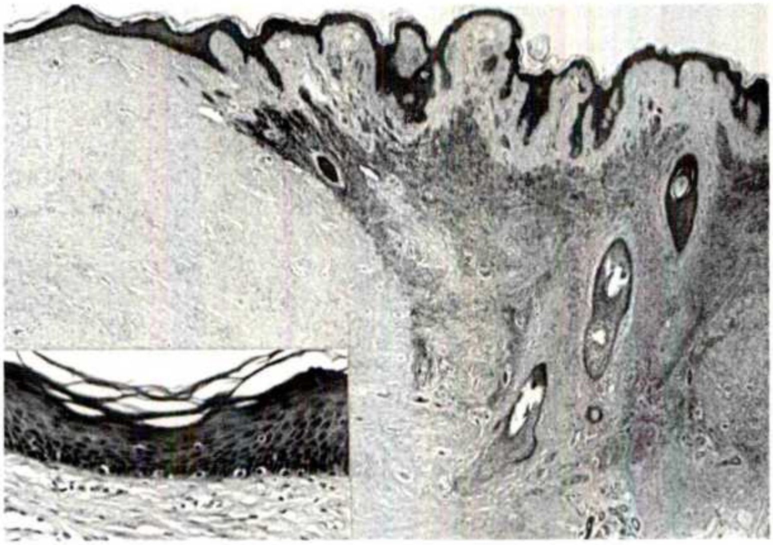

FIGURE 1. Compound naevus with scar of previous biopsy on the left. There is no increase in melanocyte number and no atypia of basal melanocytes (see inset) in the epidermis overlying the scar.

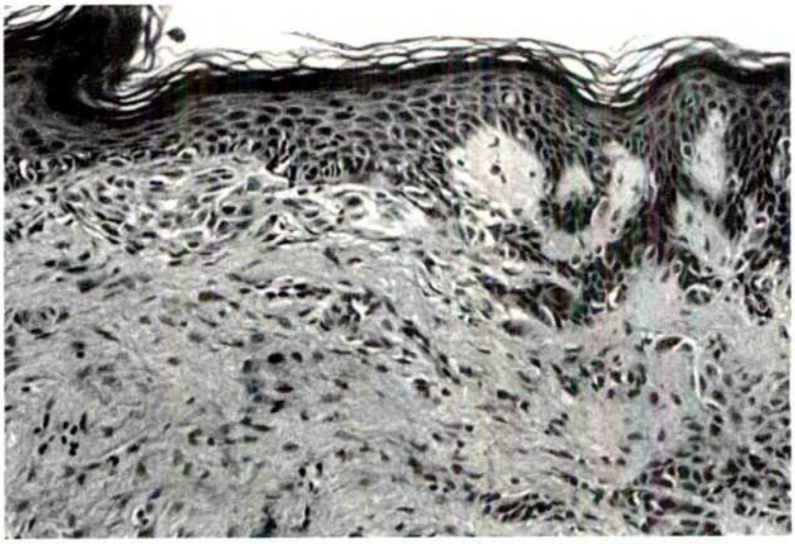

FIGURE 2. Part of a compound naevus with a scar from an incisional biopsy to left and centre. There is an increase in the number of basal melanocytes over the scar, which also exhibit some atypia. However, the features are not likely to be confused with melanoma.

DISCUSSION

There are several reasons why it might be desirable to perform incisional biopsies of benign melanocytic naevi. Dysplastic and congenital naevi are considered potential precursors of melanoma[4–7] but they are by no means obligate precursors, since only a very small minority of such lesions progress to melanoma.[1,8] Steijlen *et al.*[8] investigated congenital naevi and concluded that co-expression of two or more progression-associated antigens might indicate malignant potential. They recommended performing several incision biopsies of giant congenital naevi to identify lesions at risk.

The malignant potential of individual dysplastic naevi may be related to histological variations such as the degree of cytological atypia. If incisional biopsy is safe then this would allow easy sampling of many more naevi in a patient with numerous clinically atypical moles. This histological information could then be used in conjunction with the clinical features and family history to decide the optimum management of an individual patient. Serial biopsy of dysplastic naevi would allow us to observe their natural history and test the hypothesis of maturation.

In this study we have shown that incisional biopsy of melanocytic naevi does not produce the atypical features described after shave excision and does not affect the histology of the residual lesion. We suggest that this allows investigation of congenital and dysplastic naevi without removal of the entire lesion, and permits serial biopsies of individual melanocytic lesions.

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Eczematous infiltrated plaques to subcutaneous heparin: a type IV allergic reaction

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SUMMARY

Two patients are described who had delayed reactions with eczematous plaques to subcutaneous injections of heparin. Subcutaneous challenge with high and low molecular weight heparin compounds were positive in both patients. Patch and intradermal tests with heparin were positive in one subject. Histological and immunohistochemical studies indicated that it was a type IV allergic reaction.

Heparin is a potent inhibitor of blood clotting and is used in low doses for the prevention, and in high doses for the treatment, of thromboembolic disorders. Although the chemical structure is known, the heparins that are used are derived from animal tissue such as porcine intestinal mucosa or from bovine lung. High molecular weight compounds average 12–20 kDa, whereas the low molecular weight heparins are in the range of 4–6 kDa.

Haemorrhage is the most common of the toxic and allergic adverse effects of heparin involving the skin and mucous membranes.[1,2] Rarely, after intravenous administration allergic reactions such as rhinoconjunctivitis,[2] asthma,[3] urticaria[3–5] and anaphylaxis[5–8] have been reported. Two cases with an anaphylactoid reaction have been attributed to the preservative used in heparin.[5–8] Another rare complication is heparin-associated thrombocytopenia, of which there are two forms: one a mild and transient asymptomatic fall in the number of platelets within 2–5 days, and another in which there is severe thrombocytopenia after 6 days of treatment and which has been termed heparin-associated thrombocytopenia and thrombosis syndrome (HATT).[9] This form is the consequence of local platelet aggregation caused by antiheparin antibodies.[9–11] It may result in necrotizing skin lesions, disseminated thromboembolism and death.[1,10,11] Local skin necrosis has been reported following subcutaneous injection of heparin[1,7,9–11] and rarely following systemic administration.[1] It appears indistinguishable from coumarin necrosis.[1,12] Over the past few years there have been a number of reports of localized plaque reactions without skin necrosis occurring following subcutaneous heparin therapy (Table 1). We report two further patients who had developed eczematous plaques following subcutaneous injections of heparin.

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TABLE 1. Cases with delayed eczematous plaque reactions to heparin

Cases (*n*)	Age (years)	Sex	Heparin type	Preservative	Dose (IU/day)	Onset (days)	Patch test		Intradermal test		Further findings	Reference
							Heparin	Preservative	Heparin	Preservative		
1	45	F	Bov	—	1 × 40,000*	9	ND	ND	Pos	ND	Prick test neg, eosinophilia	13
1	26	F	—	Chlorbutol	—	5	ND	ND	Neg	Pos	MIF pos (preservative)	28
1	26	F	Porc/Na	Chlorocresol	2 × 10,000	20	ND	ND	Pos (72 h)	Neg	LMH neg	4
1	55	M	Porc	—	3 × 5000	10	ND	ND	ND	ND	Thrombocytopenia	15
3	—	—	—	Chlorocresol	Low dose	16–17	Neg	Neg	Pos (delayed)	Neg	LTT neg	16
1	60	F	Ca	None	2 × 5000	14	Neg	N/A	Pos (immediate)	N/A	Prick test neg	18
6	60–73	5F/1 M	—	—	2 × 7500	10–20	ND	ND	1 Pos (delayed), 2 neg	ND	Prick test neg, platelets normal	19
1	71	F	—	—	3 × 5000	20–28	ND	ND	ND	ND	—	23
1	35	F	Porc/bov, Na	None	2 × 5000	35	ND	N/A	ND	N/A	Bovine heparin pos	24
1	71	F	Porc, Ca	None	2 × 5000	7	Neg	N/A	Pos (immediate/delayed)	N/A	—	20
1	64	F	Porc, Ca	None	3 × 5000	3	Neg	N/A	Pos (72 h)	N/A	LMH pos	22
1	39	F	Porc, Na	Chlorocresol	2 × 5000	3	Pos	Neg	Pos (immediate/delayed)	Neg	Other heparins pos	21
1	65	F	Porc, Ca	None	2 × 5000	7	Neg	N/A	ND	N/A	Prick test pos	19
1	29	F	—	—	—	> 5	ND	ND	ND	ND	LMH neg, eosinophilia	25
15	—	14F/1M	Diverse	Chlorocresol	2–3 × 5000	3–21	3/14 pos	ND	2/7 pos (delayed)	ND	Heparin s.c. pos, platelets normal	26

Pos, positive. Neg, negative. N/A, not applicable. ND, not done. Porc, porcine heparin. Bov, bovine heparin. LMH, low molecular heparin. —, not mentioned. *, single dose intramuscular.

CASE REPORTS

Case 1

This 49-year-old lady presented with erythematous, itchy plaques on both thighs that had developed in the previous week. She had been treated with antihistamine drugs without any improvement and was referred because of a suspected cellulitis. Three weeks earlier she had a hysterectomy and during her stay in hospital she had received 5000 IU of heparin (Liquemin®) twice daily to prevent thromboembolism. On examination, there were inflammatory infiltrated lesions on both thighs that were not tender or painful (Fig. 1). A skin biopsy revealed marked oedema and a dense perivascular infiltrate in the upper and mid-dermis. An adverse reaction to Liquemin® was suspected, and she was treated with a topical corticosteroid. The plaques cleared within 2 weeks.

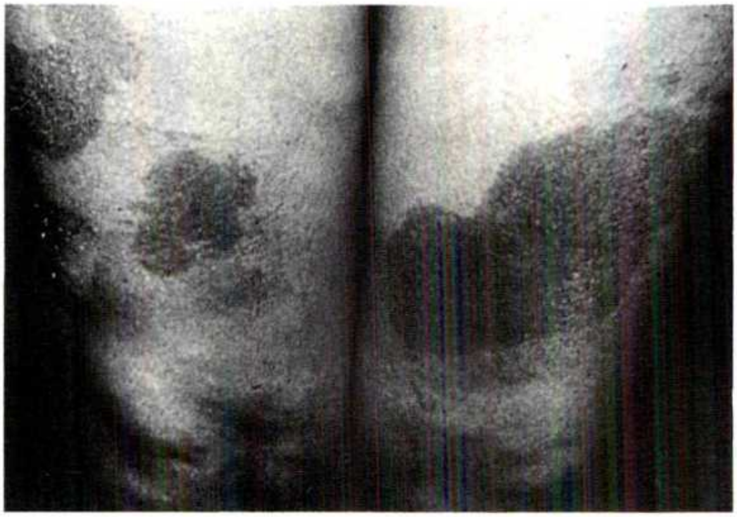

FIGURE 1. Case 1. Infiltrated plaques on both thighs 2 weeks after subcutaneous heparin injections.

Case 2

This 49-year-old male patient had a vertical banded gastroplasty for an otherwise untreatable obesity. Because of varicose veins and previous thromboses, he had been on coumarin therapy for several years. He had in the past been treated with heparin without any side-effects and he had no history of allergy or of other skin disorders. Five days before surgery he was treated with 10,000 IU heparin (Liquemin®) three times a day which was injected into the abdominal wall. At the injection sites, 4 days later, he developed pruritic, infiltrated plaques. Within 2 days these plaques became confluent and he developed an acute eczematous dermatitis (Fig. 2). A diagnosis of an allergic reaction to Liquemin® was made and the patient was then treated with a low molecular weight compound (Low Liquemin®) 2500 IU once a day for 3 days. This was injected into the thighs and after 3 days there developed further infiltrated plaques. Because of these reactions, treatment with heparin was discontinued and he was then anticoagulated with coumarin. The eczematous dermatitis cleared with topical steroids.

Allergy tests

These included skin prick, intradermal, subcutaneous and patch tests with the usual routine

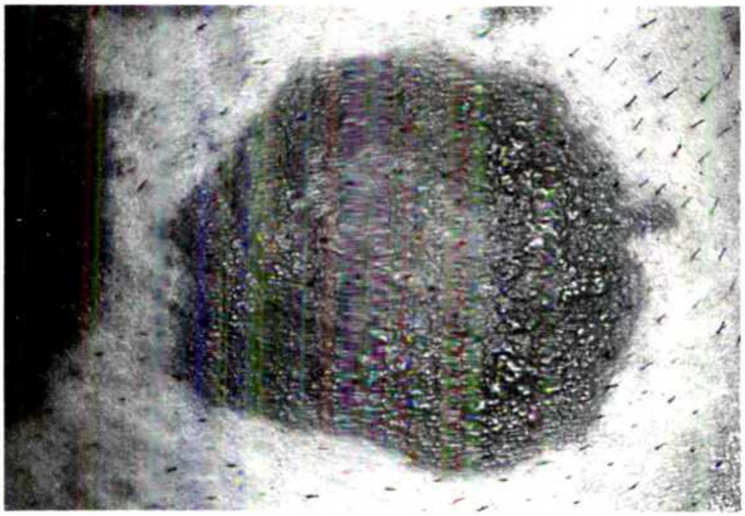

FIGURE 2. Case 2. Acute eczematous dermatitis 5 days after subcutaneous heparin injection.

allergens, single components of Liquemin® and the commercially available preservative-free, fragmented low molecular weight heparins (Fraxiparine®, Low Liquemin® and Sandoparin®). All the tested heparins were of porcine origin.

Biopsies

Skin biopsies were taken from infiltrated plaques and from test sites for routine histology and sections were stained with haematoxylin and eosin. In addition, portions of the biopsies from Case 2 were snap frozen in liquid nitrogen and stored at −20°C. Sections were cut in a cryostat and a panel of monoclonal antibodies was used for these immunohistochemical studies to characterize the inflammatory cell infiltrate in the skin lesions using standard techniques. The monoclonal antibodies used included Leu 4/CD3 (T cells), Leu 2a/CD 8 (suppressor/cytotoxic T cells), Leu 3a/CD 4 (helper–inducer T cells), Leu 6/CD1 (Langerhans cells), Leu 14/CD 22 (B cells), Leu 11b/CD 16 (natural killer cells), IL-2/CD 25 (interleukin-2 receptor), HLA-DR (MHC class II antigen). For the controls, the primary antibodies were omitted.

Laboratory investigations

These included a full blood count including platelets, prothrombin levels and blood chemistry. Complement levels and total IgE were measured and a platelet aggregation test with heparin and a lymphocyte transformation test with the single components of Liquemin® were also performed.

RESULTS

Allergy tests

In Case 1, immediate and late readings of the prick tests and intradermal tests as well as the patch tests were negative. Subcutaneous challenge with pure heparin, both Liquemin® and Fraxiparine® resulted in erythematous plaques within 48 h reaching a maximum after several days. The other components of Liquemin® were negative.

In Case 2, immediate reactions of the prick and intradermal tests with routine allergens and

TABLE 2. Test results of heparin compounds and components

		Test (reading time)							
		Prick (15 min)		Intradermal (15 min)		Patch (48 h/96 h)		Subcutaneous (72 h/120 h)	
Agent	Concentration	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2
Heparin	20,000 IU/ml	—	—	—	—*	—	+ +/+ + +	Plaque	Plaque, vesicles
Na-pyrosulphite	1 mg/ml	—	—	—	—	—	—	—	—
p-chloro-*m*-cresol	0·8 mg/ml	—	—	—	—	—	—	—	—
Distilled water	As is	+	—	—	—	—	—	—	—
Fraxiparine®	3075 IU/0·3 ml	—	—	—	ND	ND	+ +/+ + +	Plaque	Plaque, eczema
Low Liquemin®	2500 IU/0·2 ml	ND	ND	ND	ND	ND	+ +/+ + +	ND	Plaque, eczema
Sandoparin®	3000 IU/0·5 ml	ND	ND	ND	ND	ND	ND	ND	Plaque

* + +/+ + at 48 h/96 h. ND, not done.

single components of Liquemin® were negative, but a late reaction developed at 48 h at the intradermal test site of heparin. Subcutaneous challenge with the single components and low molecular weight heparins revealed positive late reactions. Patch tests to heparin, Fraxiparine® and Low Liquemin® were positive (Table 2).

The skin prick and patch tests with routine allergens were negative in both patients.

Histological studies

In Case 1 the biopsy from an early plaque revealed marked oedema and a dense perivascular lymphocytic infiltrate in the upper and mid-dermis. There was spongiosis of the epidermis, and oedema with a perivascular mononuclear cell infiltrate of the upper dermis. In Case 2 the biopsy showed an eczematous reaction with spongiosis of the epidermis and oedema with a perivascular mononuclear cell infiltrate of the upper dermis. There was no evidence of a vasculitis (Fig. 3).

Immunohistochemical studies

Most of the cells of the perivascular infiltrate in the upper dermis were Leu 4-positive T cells. Leu 3a-positive T cells predominated in the dermis and were approximately 80–90% of the perivascular cellular infiltrate (Fig. 4). Only 10–20% of the cells reacted with Leu 2a. Helper T cells and suppressor–cytotoxic cells were also recognized among the cells infiltrating the epidermis and in the intraepidermal vesicles. Leu 6 and HLA-DR-positive dendritic cells were present in the epidermis and in close apposition to T cells in the intraepidermal vesicles. Leu 6-positive cells were present in the upper dermis. Most of the T cells within the dermal infiltrate were HLA-DR positive. A few epidermal keratinocytes expressed HLA-DR. Also there were T cells expressing interleukin-2 receptor in the intraepidermal vesicles. Only a few Leu 14-positive B cells could be identified in the dermal infiltrate.

Laboratory investigations

In both patients, the routine laboratory tests were normal. Lymphocyte proliferation and platelet aggregation tests were negative in Case 1. In Case 2, possibly due to preservation of the sample in heparinized tubes, a significant lymphocyte proliferation was observed in all the samples, including the control. Also, platelet aggregation was present in platelet-enriched serum samples following the addition of high and low molecular weight heparins. No such reaction was seen with the patient's serum and donor platelets, indicating the presence of

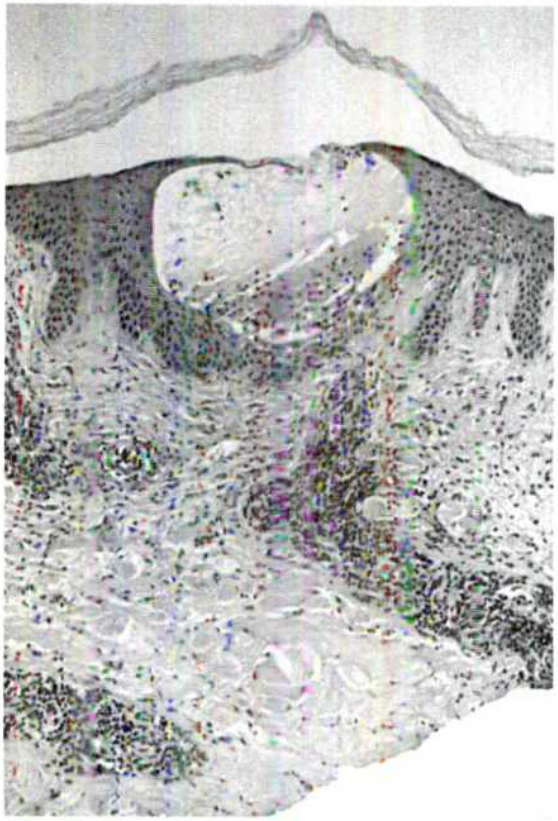

FIGURE 3. Spongiotic vesicle, dermal oedema and perivascular mononuclear infiltrate of the upper dermis.

specific platelet-bound antiheparin antibodies. Since the patient had normal platelet counts throughout the heparin treatment and the test period, the clinical relevance of the finding was not established.

DISCUSSION

The first report of a plaque reaction to heparin was in 1952.[13] In this case treated with intramuscular bovine heparin there were positive intradermal tests and a delayed reaction at previous injection sites. A similar reaction to subcutaneous heparin has been observed in several patients in whom the plaques resolved after several weeks despite continuation of therapy.[4] Also a patient was described who developed tender plaques at injections sites of which one became necrotic (heparin-induced necrosis).[14] A further case[15] we believe to be of an eczematous plaque reaction. Only recently have delayed plaque reactions been clearly differentiated from heparin-induced skin necrosis.[2,16] Erythematous plaques developed several days following the initiation of subcutaneous heparin therapy and were followed by eczematous changes.[16] Intradermal tests with heparin were positive at 48 h and these reactions resembled those to tuberculin-type IV delayed hypersensitivity. A lymphocyte transformation test was negative in three cases.[16] In a further report of six patients the plaque reaction was considered to be a mild form of heparin necrosis, though delayed hypersensitivity has also been suggested.[17] Further patients with similar clinical features and with either positive, immediate[18,19] or delayed tests[20–22] and three

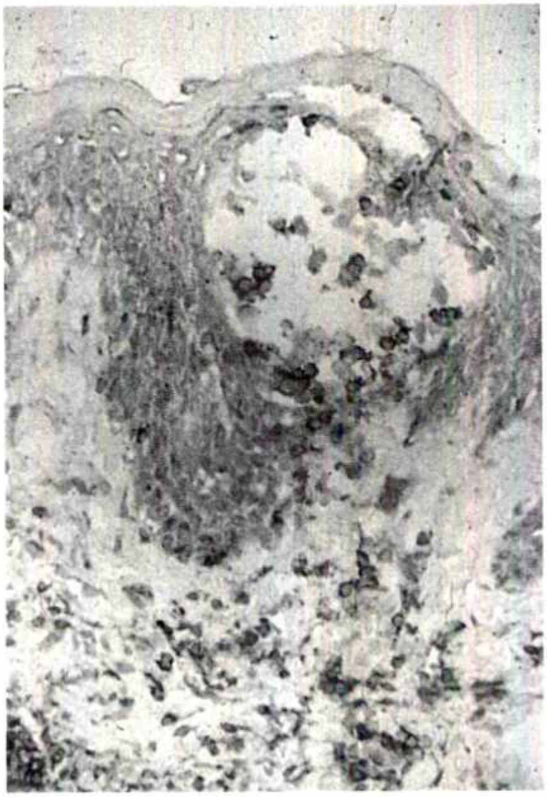

FIGURE 4. Helper–inducer T cells (Leu 3a/CD4 positive) constitute 80–90% of the cellular infiltrate in the dermis and within the spongiotic vesicle.

cases that were not tested[23-25] have been reported (Table 1). In a recent report of 15 patients, the eczematous lesions started 3–21 days following initiation of heparin therapy[26] and in 14, subcutaneous provocation tests with various heparins were positive. Histological and immunohistochemical studies of three lesions suggested that the reaction was a delayed type of hypersensitivity.

Our two patients had the same clinical features, with a delayed onset of infiltrated eczematous plaques at the injection sites. Histological findings were similar to those reported.[15,17,21,22,26] The predominance of helper T cells and HLA-DR positive Langerhans cells in the intraepidermal vesicle also supports a type IV allergic reaction.[27] A subcutaneous injection of pure heparin resulted in an eczematous reaction in both patients and in Case 2, patch and intradermal tests with pure heparin were also positive. A plaque reaction to other components in heparin such as preservative is rare[26,28] and this has been excluded in our patients.

There was *in vitro* platelet aggregation in Case 2. Platelet aggregation has been shown to be due to circulating antibodies directed towards heparin-coated platelets and resulting in severe thrombocytopenia.[9,29] In our case, we had evidence for platelet-bound antiheparin antibodies, but without any clinical consequence. Perhaps the treatment period of 7 days was too short to elicit an adverse reaction.

The heparin antigen responsible for these plaque reactions has as yet not been identified. Protein impurities are unlikely, since most of the plaque reactions occurred to heparins from different manufacturers. There was also no relationship between the occurrence of the plaques and the molecular weights of the heparins or their animal source. With a few exceptions,[2,25] all the cases tested so far reacted to both high and low molecular weight heparins[17,20,22,26] and in one patient positive reactions to both porcine and bovine heparins were observed.[24] It has been proposed that the eczematous infiltrated plaques result from the binding of the low allergenic carbohydrate heparin molecule to a dermal protein.[26] In patients with suspected plaque reactions heparin therapy should cease and subcutaneous provocation tests should be done.[26]

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Regressing atypical histiocytosis and lymphomatoid papulosis: variants of the same disorder?

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SUMMARY

We report a patient with lymphomatoid papulosis who developed a lesion with the clinicopathological features of regressing atypical histiocytosis. Immunohistochemical studies supported a T-cell histogenesis and many of the atypical cells demonstrated BerH2 (Ki-1 antigen) positivity. The case supports the view that regressing atypical histiocytosis and lymphomatoid papulosis are different manifestations of the same disease spectrum.

CASE REPORT

An 8-year-old Caucasian male originally presented in 1975 with an asymptomatic, self-healing papular eruption over the previous 3 years. A clinical diagnosis was made of pityriasis lichenoides. He presented later the same year with a 3-month history of a large, nodular, ulcerative lesion over the left anterior superior iliac spine suggestive of a high grade malignancy. There was an elevated edge and some secondary infection in the ulcer crater which measured 4 cm in diameter (Fig. 1). He had no other symptoms of lymphoma such as weight loss, night sweats or pruritus and in particular no evidence of lymphadenopathy or hepatosplenomegaly. Routine laboratory investigations, including full blood count, blood film, ESR and bone marrow examination were normal. Lymphangiography showed only features suggestive of a reactive hyperplasia of lymph nodes.

Histopathology of the ulcer revealed pseudoepitheliomatous hyperplasia at the ulcer margin (Fig. 2), and a dense atypical mononuclear cell infiltrate throughout the dermis reaching the subcutaneous fat. There was no evidence of vascular invasion (Fig. 3). The infiltrate was pleomorphic with cells demonstrating hyperchromatic nuclei and numerous atypical mitotic figures consistent with a high grade lymphoma (Figs 3 and 4). No Reed–Sternberg cells or cells demonstrating erythrophagocytosis were observed.

The ulcer was treated with radiotherapy (4000 R in eight fractions over 17 days) and after several weeks resolved completely. In 1976 another ulcer, measuring 4 × 1·5 cm developed in the

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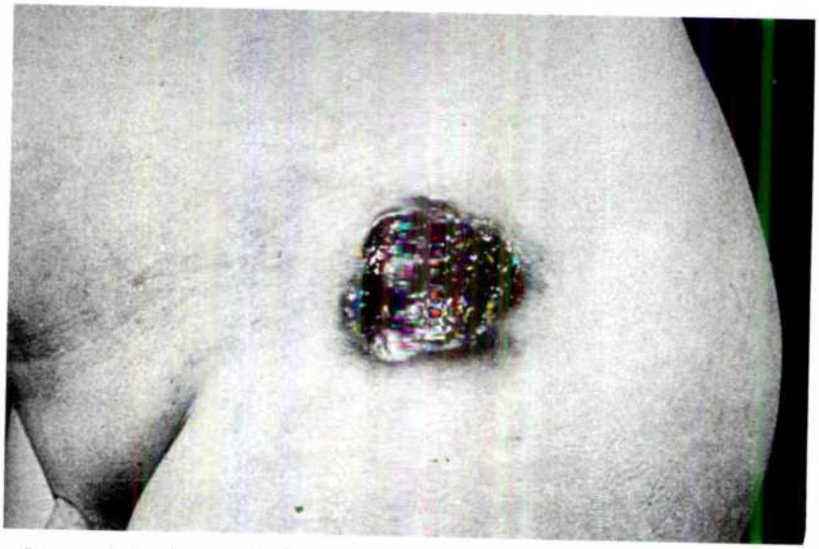

FIGURE 1. Large nodular ulcerative lesion measuring 4 cm in diameter over left hip in patient aged 8 years.

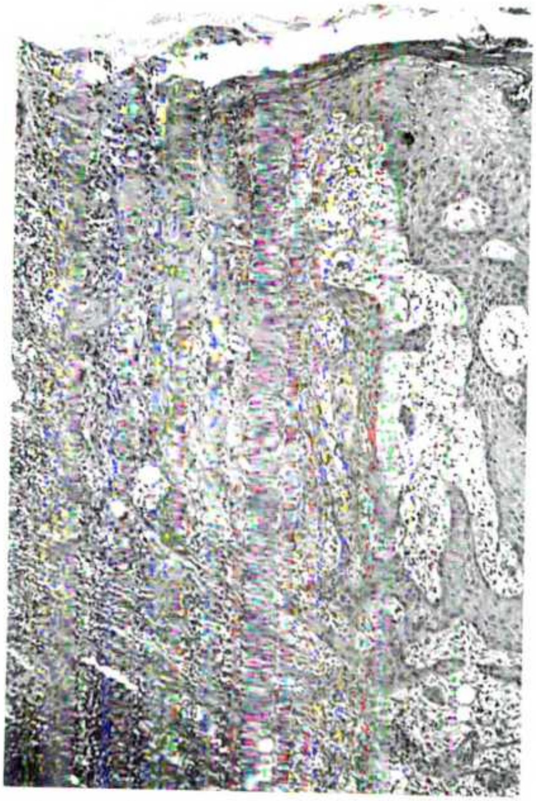

FIGURE 2. Histology from ulcer margin shown in Figure 1 demonstrating a dense polymorphous cellular infiltrate throughout the dermis with prominent pseudoepitheliomatous hyperplasia (H&E × 100).

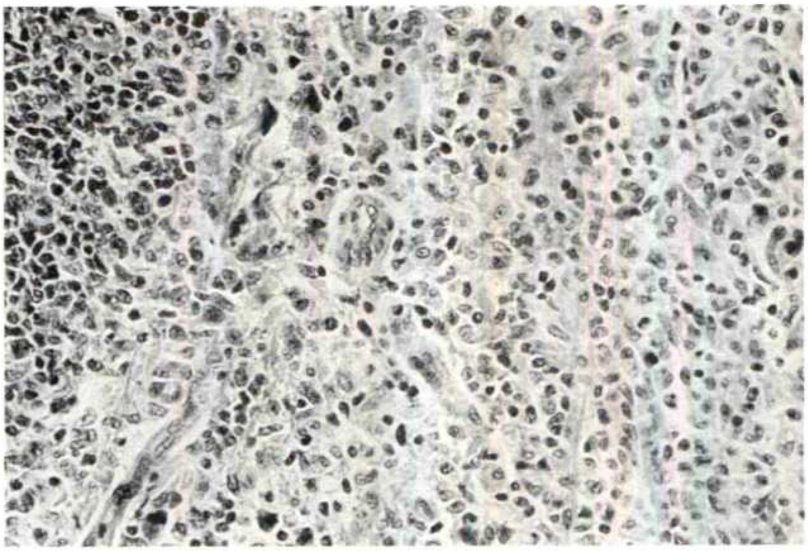

FIGURE 3. Dense dermal infiltrate of atypical, predominantly lymphoid cells with no evidence of vascular invasion (H&E × 200).

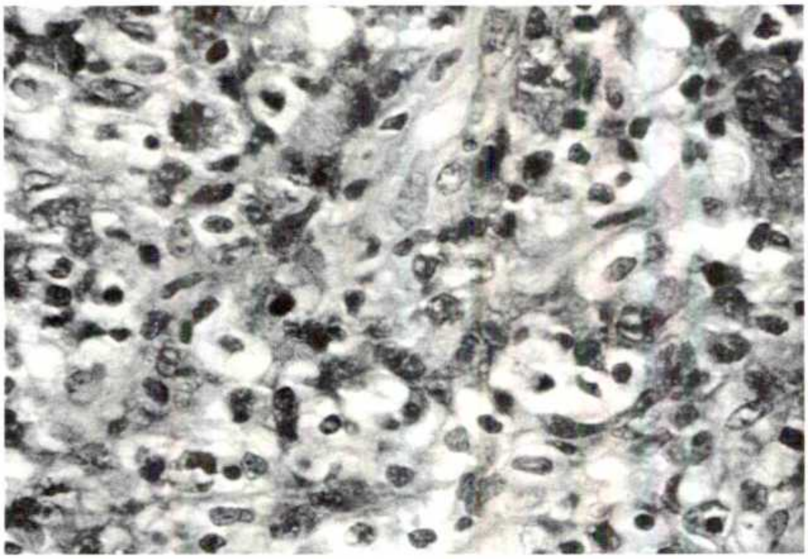

FIGURE 4. High power histological detail of the ulcer demonstrating a pleomorphic, polychromatic infiltrate with atypical mitoses suggestive of a high grade lymphoma (H&E × 750).

right groin. This was also treated successfully with 3500 R in seven fractions over 15 days. The patient subsequently developed a widespread papulonodular eruption, the lesions of which occasionally became necrotic, ulcerated and healed with scarring usually within 6 weeks. Three further skin biopsies showed an atypical wedge-shaped lymphoid infiltrate with prominent exocytosis of lymphocytes into the epidermis. The cells had hyperchromatic convoluted nuclei

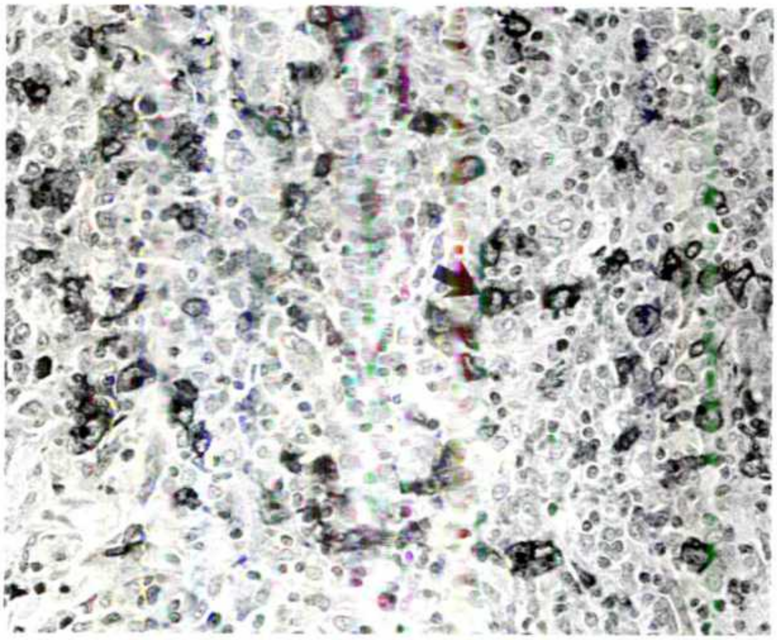

FIGURE 5. Numerous mononuclear cells including atypical cells were positively labelled (arrow) with the T-cell marker UCHL1 (Haemalum × 250).

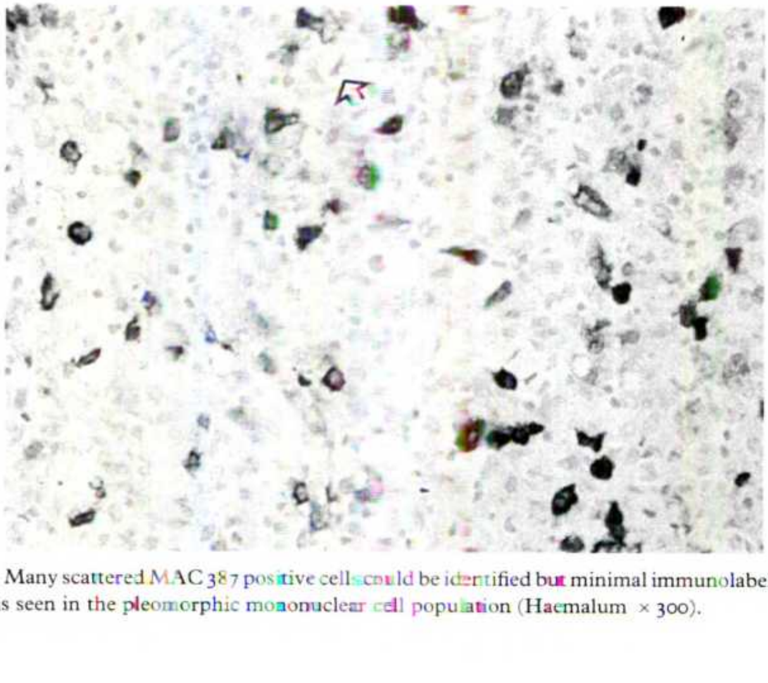

FIGURE 6. Many scattered MAC 387 positive cells could be identified but minimal immunolabelling (open arrow) was seen in the pleomorphic mononuclear cell population (Haemalum × 300).

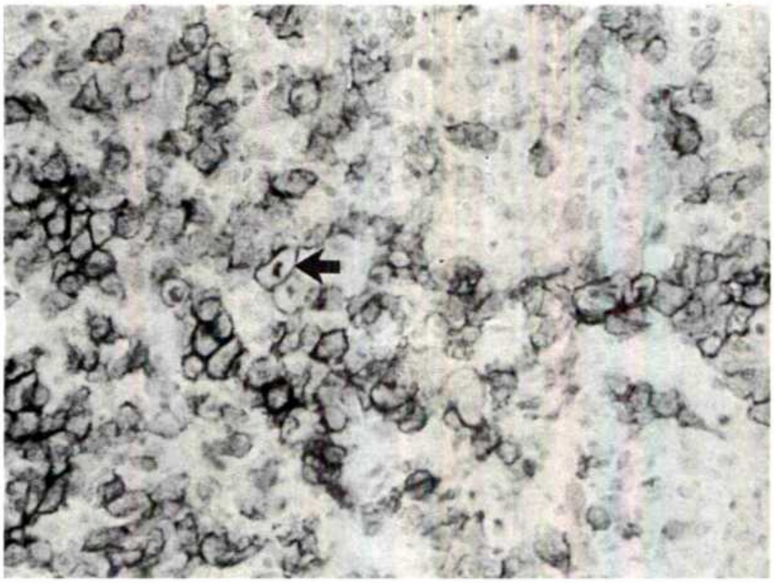

FIGURE 7. Many atypical cells expressed the membrane bound Ki-1 antigen using the monoclonal antibody BerH2 (arrow), including those with atypical mitoses (Haemalum × 300).

mingled with large atypical mononuclear cells. There was overlying epidermal thickening. The clinicopathological features were now consistent with lymphomatoid papulosis (type A). The condition remained active for several years but has been in complete remission since 1985.

Immunophenotypic studies

Sections from all four biopsy specimens were re-evaluated both histologically and immunophenotypically. Employing a routine indirect immunoperoxidase technique in paraffin sections, the following monoclonal antibodies were used: UCHL1 (T lymphocytes–72% CD4),[1] L26 (B lymphocytes),[2] LN1 (follicular B cells),[3] MAC 387 (monocyte derived macrophages, L1 antigen),[4] and BerH2 (Ki-1 antigen [CD 30] positive cells).[5] All reagents were obtained from Dakopatts, Glostrop, Denmark, except for LN1 and LN2, which were obtained from Biotest (UK) Ltd, Birmingham, U.K.

The predominant mononuclear cells, including many atypical cells, were of the T-cell phenotype (Fig. 5). Only scattered B cells could be identified. No LN1 positivity was seen. Many MAC 387 positive cells were identified (Fig. 6). The atypical pleomorphic cells were not labelled with MAC 387 but were BerH2 positive (Fig. 7).

DISCUSSION

Regressing atypical histiocytosis (RAH) was a term first introduced by Flynn *et al.* who reported two cases of a cutaneous nodulo-ulcerative proliferation of atypical histiocytes.[6] This was based on the light microscopic, ultrastructural, surface markers and enzyme cytochemical features. Cytogenetic analysis showed aneuploidy despite the spontaneous regression of the lesions and a benign biological course. These authors interpreted RAH cells as having histiocytic differentiation, based on erythrophagocytosis, a strong reaction for FC–IgG and complement receptors in the absence of E-rosette formation. However, neither peroxidase nor cytoplasmic lysozyme nor α-1-antitrypsin was found in these RAH cells. More recently a further case was described[7] in which immunohistochemical studies suggested a T-cell lineage with Ki-1 antigen positivity in many atypical cells. Flow cytometry confirmed aneuploidy and there was rearrangement of the T-cell receptor β- and γ-chain gene which supported the T-cell origin of this tumour. A recent review of ten cases of RAH, including Flynn's original two cases has shown that the clinicopathological features of these cases are very variable.[8] Lesions could be single or multiple, often on the extremities, not associated with lymphadenopathy and could affect any age group (age range 10–80 years). Unusual histological features included large atypical mononuclear cells, epidermal erosion, and a polymorphous infiltrate. Other common features described were atypical granulation tissue, perilesional fibrosis, focal tumour cell necrosis, eosinophils and occasional pseudoepitheliomatous hyperplasia. There was no evidence of vascular invasion in any case. To date, follow-up on eight cases has shown progression to T-cell lymphoma in three cases including the two originally described by Flynn *et al.*[6] This questions the benign natural history originally suggested for RAH.

It was considered[8] that there was little difference between RAH and the nodulo-ulcerative (type A) form of lymphomatoid papulosis,[9] except for the greater degree of cell atypia in RAH. Also there may be a greater risk in these patients of developing lymphoma and so such cases require careful follow-up. We have shown that a lesion fulfilling all the clinicopathological features of RAH can occur in a patient known to have lymphomatoid papulosis. There is increasing evidence that the infiltrate in lymphomatoid papulosis has a T-cell phenotype and genotype.[10] Several authors have shown that the atypical cells of lymphomatoid papulosis often co-express pan T and Ki-1 antigens.[5,10-12] Our studies support the increasingly accepted concept that like lymphomatoid papulosis (types A and B), RAH is of T-cell lineage. We consider that this case provides further evidence that RAH represents a pleomorphic subset of nodulo-ulcerative (type A) lymphomatoid papulosis.

To continue to use the term RAH is not only misleading but confusing, particularly with regard to its histogenesis. We feel that both RAH and lymphomatoid papulosis lie on a continuous spectrum of *initially* indolent T-cell lymphomata. Perhaps RAH should be more accurately renamed pleomorphic giant lymphomatoid papulosis?

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Hereditary woolly hair with ocular involvement

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SUMMARY

A case of hereditary woolly hair associated with Coats' disease of the eyes, a rare type of exudative retinitis, is reported.

Hereditary woolly hair is a rare disorder characterized by tight curly hair which is present from birth or the first few months of life.[1] In a few published cases it has been associated with abnormalities of the skin, eye and ears. A previously undescribed association between hereditary woolly hair and Coats' disease of the eye is reported.

CASE REPORT

A 37-year-old female had tightly curled scalp hair since birth. The hair was dark and soft to touch. Her hair was kept very short and she wore wigs. Her eyebrows had always been fine. From birth she had had no vision in her left eye due to a subluxed cataract. At 21 years of age Coats' disease of the eyes, a rare exudative retinitis, was diagnosed. Nine years later a subcapsular cataract was diagnosed in the right eye and was removed 2 years later.

Family history

Woolly hair was manifested in at least one individual per generation suggesting an autosomal dominant inheritance pattern (Fig. 1). No other family member had any ophthalmological problem.

Examination

The patient was Caucasian with tightly coiled soft scalp hair. Her eyebrows and eyelashes comprised fine vellus hair and she had no axillary or pubic hair. Her nails were normal. Macroscopically the scalp hair curl diameter was 0·5 cm and microscopically the hair structure was normal. On ophthalmological examination the left eye showed no perception of light and there was a complicated subluxed cataract and detached retina due to massive exudates. The right eye was aphakic and visual acuity was 6/24. The retina was scarred from cryopexy and argon laser treatment. Heavy exudate formation was present.

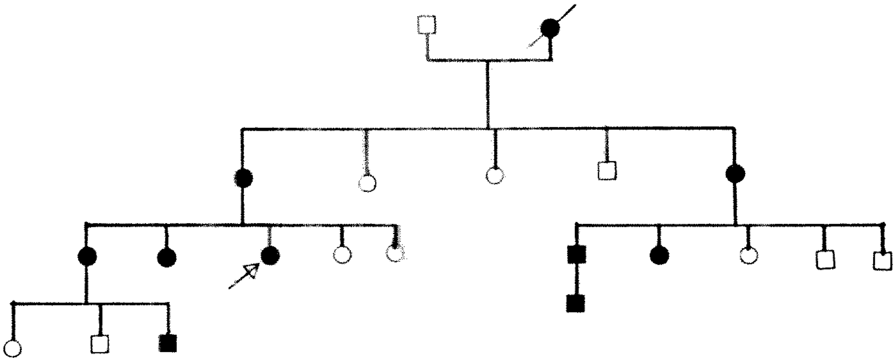

FIGURE 1. Family pedigree—hereditary woolly hair.

DISCUSSION

Hereditary woolly hair with autosomal dominant inheritance is one subgroup of woolly hair disease. The two other subdivisions are familial woolly hair, an autosomal recessive condition, and woolly hair naevus. In a few cases it has been associated with abnormalities of the hair shaft, i.e. trichorrhexis nodosa, pili torti and pili annulati.[1] Keratosis pilaris atrophicans with or without Noonan's syndrome has also been described.[2] These features were not present in this case. In one previous study of eight patients with hereditary woolly hair no abnormalities of the hair were noted at other sites apart from the scalp, in contrast to the present case who had vellus eyelashes and eyebrows and absent axillary and pubic hair.

Associated ocular abnormalities have previously been reported. A family with hereditary woolly hair and cataracts has been described[3] in which a father and three of his eight sons were affected with the remaining sons having normal hair and eyes. Woolly hair naevus has been described with persistent pupillary membrane in one case.[4] A separate case exhibited pupillary membrane remnants, congenital paralysis of the superior oblique muscle, cataracts and focal retinal dysplasia.[5]

The patient described here is the only member of her family to have eye problems. She has cataracts and Coats' disease of the eye. This rare disease of unknown aetiology is characterized by haemorrhages, exudative retinal lesions and occasionally detached retina and glaucoma. The complete spectrum of reported manifestations occurred in this patient. Coats' disease exhibits no distinct genetic pattern of inheritance but familial clustering has been reported.[6]

Several different eye disorders have now been described in association with woolly hair disease. It is therefore prudent for new cases of woolly hair disease to undergo ophthalmological examination, as early therapy may prevent loss of vision.

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Squamous cell carcinoma complicating hidradenitis suppurativa

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SUMMARY

We describe a patient who suffered over 40 years from persistent, extensive hidradenitis suppurativa (HS) and who developed squamous cell carcinomas at two different sites.

Hidradenitis suppurativa (HS) most commonly affects the skin of the groin and perineum, axillae and less commonly the buttocks and upper thighs. It results in fibrosis, sinus formation and scarring. In common with other chronic inflammatory skin conditions, it is rarely complicated by squamous cell carcinoma (SCC). So far there are 15 such cases reported in the literature.

CASE REPORT

A 67-year-old Caucasian man was referred to us with a 40-year history of multiple discharging sinuses involving the buttocks and groins. His skin condition had deteriorated markedly over the previous 12 months. Physical examination revealed an obese man of 95 kg with severe HS involving the buttocks, and perianal and perineal skin, extending onto the upper inner thighs and inguinal skin. There were multiple abscesses and suppurating, discharging sinuses as well as extensive scar tissue from healed lesions.

Investigations

Haematological investigations revealed a mild iron deficiency anaemia with a haemoglobin of 10·2 g/dl, MCV of 67 fl and an iron deficient picture on the blood film. The white cell count ranged between 13·1 and 15·6 × 10^9/l, usually with a moderately raised neutrophil count. The erythrocyte sedimentation rate was elevated from 81 to 90 mm/h. Renal and liver function tests were normal. Immunoglobulins were diffusely raised with IgA 11·4 g/l, IgG 24 g/l and IgM 1·6 g/l. Complement studies revealed a low C4 at 0·13 g/l with a normal C3 at 0·90 g/l. Rheumatoid factor (RA latex) was positive at 1 in 8.

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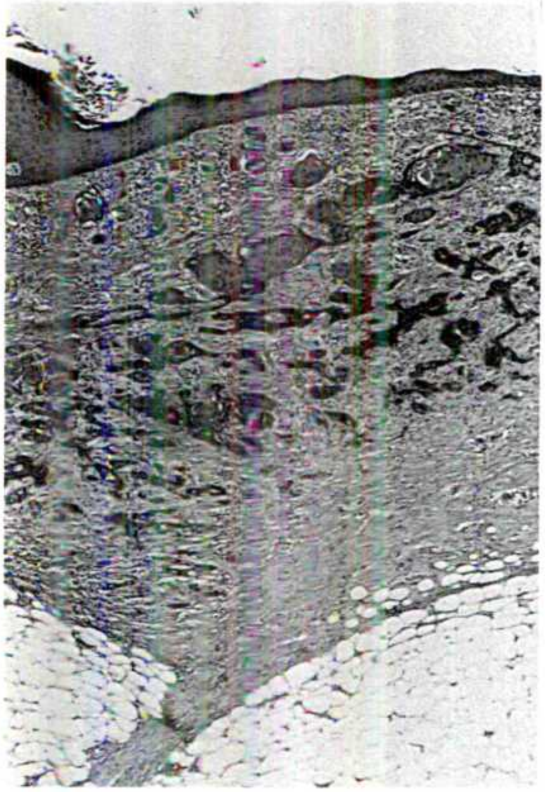

FIGURE 1. Biopsy of sacral ulcer showing infiltrating, well to moderately differentiated squamous cell carcinoma (Haematoxylin and eosin × 132).

Microbiology. Skin swabs taken from suppurating lesions during the first 6 months cultured five main pathogenic organisms: anaerobic *Streptococcus*, *Proprionobacterium acnes*, *Bacteroides corrodens*, *Bacteroides melaninogenicus* and *Bacteroides fragilis*. All five organisms were sensitive to metronidazole and amoxycillin with clavulanic acid.

Histology. A skin biopsy taken from the inguinal and buttock hidradenitis showed a heavy mixed inflammatory reaction in the dermis extending to the subcutaneous tissues. Occasional foreign body giant cells were present and the findings were compatible with the clinical diagnosis of HS. A biopsy of an indolent sacral ulcer in September 1984 revealed an infiltrating, well to moderately differentiated squamous carcinoma (Fig. 1). The tumour cells extended through to the deep dermis and in some areas involved the subcutaneous tissues. A second area of non-healing chronic ulceration of the left hip was biopsied in January 1985 and showed a moderately differentiated squamous cell carcinoma of the skin. The tumour extended to the deep dermis, but did not involve the subcutaneous tissues.

Radiology. Chest X-ray was normal. A barium enema was performed to investigate the mild anaemia, and to exclude the possibility of occult Crohn's disease contributing to the inguinal and perineal fistulae; it showed diverticulosis but was otherwise normal.

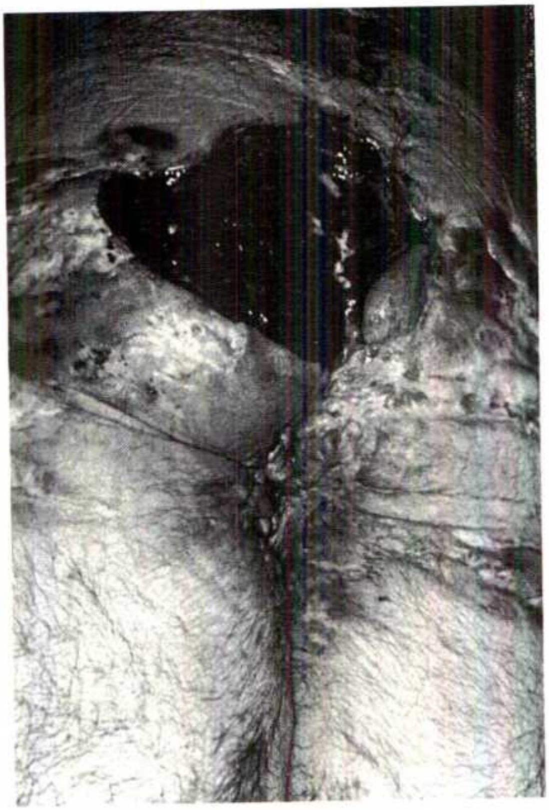

FIGURE 2. Squamous cell carcinoma over the sacrum.

Treatment and progress

The patient was admitted to hospital for treatment which included surgical incision and the marsupialization of five sinuses. The remaining hidradenitis was treated by cleaning with an antiseptic twice daily and a 4-week course of antibiotics. During the next 2 years he required frequent admission to hospital, treating in rotation each involved area. His condition had improved until the development of an indolent non-healing area over the sacrum (Fig. 2). A biopsy revealed a squamous cell carcinoma which was treated by deep excision using a diathermy loop. The large ulcerated area was then pinch grafted, which initially appeared to take, but which was later rejected. Further biopsies from the granulation tissue revealed a recurrence of the squamous cell carcinoma. In January 1985 he developed a new area of what appeared to be granulation tissue in a different area of hidradenitis suppurativa over the left hip. A biopsy confirmed a second squamous cell carcinoma. His general health deteriorated rapidly and he died with bronchopneumonia in February 1985, 2 years after the initial referral. A post mortem examination was not performed.

DISCUSSION

Hidradenitis suppurativa (HS), acne conglobata and dissecting periofolliculitis of the scalp are three similar diseases which are referred to as the 'follicular occlusion triad'. Squamous cell carcinoma (SCC) has been reported as a late complication in three conditions.[1-3]

Despite being a relatively common skin disease, hidradenitis suppurativa is rarely compli-

TABLE 1. Reported cases of hidradenitis suppurativa complicated by squamous cell carcinoma (SCC) of the skin

Author		Case no.	Age (years)	Sex	Site of SCC	Metastases	Years HS before diagnosis of SCC	Complications	Outcome
Jackman[4]	1959	1 2 3 4	Limited clinical details given Delay of 1–4 years before SCC diagnosed				19–32 years		1 death from widespread metastases. Apparent cure of three patients after wide local excision
Donsky and Mendelsone[5]	1964	5	44	M	Buttocks	No	23	Local recurrence despite extensive surgery and DXT	Uncertain
Humphrey *et al.*[3]	1969	6	48	M	Buttocks	Yes, to inguinal lymph nodes	8		? death
Gordon[6]	1977	7	28	F	Buttocks and axilla	Yes, to single left inguinal lymph node	17		Uncertain. Inguinal lymphadenopathy persists
Alexander[7]	1979	8	40	M	Thigh			Hypercalcaemia	Death
Johnson[8]	1980	9	41	F	Buttocks	Local lymph nodes inguinal pulmonary metastases	[illegible]	Sepsis	Death
		10	34	M	Buttocks	No	5		Death
Mora and Perniciaro[9]	1981	11	47	M	Perianal		3		Death
		12	54	M	Perianal		< 1		Death
Black and Woods[10]	1982	13	44	M	Buttocks	Yes	22	Deep recurrence with posterior perianal invasion requiring total proctectomy	Death
Sparks *et al.*[11]	1985	14	35	M	Buttocks	Yes, bilateral inguinal and deep iliac adenopathy. Tumour replacing muscles	10	Anaemia. Hypercalcaemia	Uncertain ? death
Zachary[12]	1987	15	55	M	R. Buttock	No.	3	None	No evidence of recurrent at 1 year follow-up
Current report	1989	16	67	M	Buttocks/ left thigh	No	40	Anaemia local recurrence	Death

cated by squamous cell carcinoma. There are 15 cases on record of which 11 have been reported in detail (Table 1). The length of time from onset of HS to diagnosis of SCC varied from less than 1 year to 32 years. In the majority of cases, the SCC have occurred in the anogenital region, with single case reports of SCC arising in the axilla and thigh. Death occurred in at least seven of the 15 cases.

The prognosis for cases of SCC occurring in HS is poor (Table 1). This may be explained by the often advanced state of the SCC at the time of diagnosis and difficulty in obtaining a diagnostic biopsy. In one case there was an 8-month delay between the development of SCC and its removal by definitive surgery.[3] In another case there was an 8-month delay between development of SCC and its surgical excision, during which time seven negative biopsies were obtained before a positive histological diagnosis was made.[5] This case illustrates the importance of repeat biopsies in any atypical non-healing ulcer occurring at a site of chronic inflammation of skin.

Squamous cell carcinomas arising in chronically scarred inflamed skin are, in general, more malignant than other SCCs arising *de novo* or on a background of sun-damaged skin, as demonstrated by their rapid growth, local aggressiveness and early metastases.[2] Surgical treatment of longstanding, extensive hidradenitis suppurativa should be considered in preference to medical management as this is likely to reduce the incidence of SCC. Long-term regular follow-up of extensive HS is important as the aggressive nature of secondary SCCs make early diagnosis and surgical intervention essential.[2] Following surgery, provision should be made for close follow-up to detect evidence of metastatic disease.

In summary, our patient had longstanding, severe and extensive hidradenitis suppurativa which we managed conservatively with a combined surgical and medical approach. This proved to be unsuccessful as he developed two separate areas of squamous cell carcinoma arising from the HS. Attempts at surgical excision were unsuccessful and the aggressive nature of the SCC was illustrated by his rapid decline and death.

ACKNOWLEDGMENT

We wish to thank Mrs B.Knott for preparing the manuscript.

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Society Proceedings

Proceedings of the 247th Meeting of the Netherlands Society for Dermatology and Venereology, Free University, Amsterdam, 27 January 1990

PRESIDENT: DR H.A.M.NEUMANN
HOSTS: PROFESSOR TH.M.STARINK
PROFESSOR R.WILLEMZE

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SUMMARIES OF PAPERS

Ki-1-positive cutaneous lymphoreticular proliferations

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The monoclonal antibody Ki-1 (as well as Ber-H2 applicable to paraffin-embedded tissue) was initially developed as a Hodgkin-specific marker. Further investigations have shown Ki-1 to be an activation marker that recognizes CD30 antigen, present not only on Reed–Sternberg cells, but also on a number of large cell lymphomas, PHA-stimulated lymphocytes and viral transformed cells. A new group of malignant lymphomas has been defined, the so-called Ki-1 positive anaplastic lymphomas,[1] that have since been included in the updated Kiel classification. In the skin, reactivity with Ki-1 is found on the large atypical cells in lymphomatoid papulosis (type A).[2] Reactivity is also present on large-cell anaplastic lymphomas, that can be found primarily in the skin. These lymphomas are often present with localized disease and have a favourable course, in contrast to the Ki-1-negative large-cell lymphomas of T-cell origin.[3]

The differentiation of lymphomatoid papulosis from Ki-1-positive lymphomas can be difficult, as with patients with persistent papules or patients with one solitary, spontaneous regressing tumour. Also, in patients with lymphomatoid papulosis, an association with, or transformation to a Ki-1 lymphoma has been described. These findings support the hypothesis that lymphomatoid papulosis, Ki-1-positive lymphomas and Hodgkin's disease are part of a spectrum, in which the clinical differences are probably related to the different immune responses of the host.

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Treatment of psoriasis in the psoriasis day care centre

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We describe the treatment of 196 patients with psoriasis at a psoriasis day care centre in Ede. The results were assessed by means of the PASI scores and by evaluation of the patient questionnaires. In an average of 28·4 treatments, the PASI decreased by 84·3%, the areas evaluated including the scalp, the axillae and the groins. The questionnaires indicated that there was a rapid relapse, but in a large number of patients only a small part of the skin was affected. Additional advantages of the psoriasis day care center were the convenience and the relatively safe treatments available.

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Fumaric acid therapy in psoriasis: a long-term retrospective study on the effect of fumaric acid combination (FAC-EC) therapy and dimethyl-fumaric acid ester (DMFAE) monotherapy

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Two forms of fumaric acid (FA) therapy are current in the treatment of psoriasis: monotherapy with dimethyl-fumaric acid ester (DMFAE), and combination therapy with DMFAE plus salts of monoethyl-FAE (FAC-EC). The first involves capsules filled with 60 mg DMFAE as semi-enteric coated granules with dosages from 1 to 4 capsules a day (mean 3). With this therapy 124 patients were treated: 99 for 6 months, 61 for 12 months, and 27 for 18 months (cumulative numbers). The FAC-EC therapy involves enteric coated tablets containing 120 mg DMFAE, 5 mg MEFAE-Mg, 3 mg MEFAE-Zn and 87 mg MEFAE-Ca with dosages of 1–4 tablets a day (mean 3). Sixty-three patients were treated: 56 for 6 months, 36 for 12 months and 19 for 18 months (cumulative numbers). After 6 months a marked improvement or complete remission was obtained in 40% of the DMFAE-treated patients versus 60% in the FAC-EC group. After 12 and 18 months results were the same in both groups (65%). After 18 months, 90 patients in the DMFAE group had discontinued therapy, which in 39 was due to an unsatisfactory response and in 37 to side-effects. In the FAC-EC group these numbers were 20, 6 and 9, respectively. The most frequent side-effects were, in both groups: nausea, stomach pain, flushing, diarrhoea and generalized malaise. Lymphopenia was the most frequent laboratory finding. These side-effects were more serious in the DMFAE-group, leading to a higher percentage discontinuing treatment. It was concluded that the better therapeutic results of the FAC-EC therapy compared with the DMFAE monotherapy in the first 6 months can probably be ascribed to the higher dosage of DMFAE in the FAC-EC tablets. Moreover it was shown that effective enteric coating diminished the severity of the side-effects of the FA-therapy.

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Does fish oil protect renal function in cyclosporin-treated psoriasis patients?

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Cyclosporin A (CyA) has been shown to suppress both humoral and cell-mediated immunity by affecting the early steps of T-cell activation. At present it is one of the most widely used immunosuppressive drugs in organ transplantation. Recent studies have also shown a marked beneficial effect in the treatment of patients with psoriasis.

One of the major problems limiting a more extensive use of CyA is its nephrotoxicity. Recent studies suggest that altered haemodynamics play an important role in CyA-induced renal dysfunction. The glomerular filtration rate (GFR) is reduced both in animal experiments and in man following CyA administration, probably due to an increased production of thromboxane A2. Histological examination of the kidney after prolonged treatment with CyA reveals tubular damage.

Recently, it has been shown that fish oil, containing high concentrations of the polyunsaturated fatty acids eicosapentaenoic acid (EPA, C20 : 5 omega-3) and docosahexaenoic acid (DHA, C22 : 6 omega-3), reduces CyA-nephrotoxicity in the rat, probably by lowering thromboxane A2 production. In addition, fish oil alone may have a limited beneficial effect on psoriasis. The observations prompted us to evaluate the effects of CyA with and without fish oil in patients with severe psoriasis, in particular with respect to renal function.

Thirteen patients with psoriasis (CyA group) received cyclosporin A alone and seven patients (CyA/EPA + DHA group) received a combination of cyclosporin A and fish oil (6 g EPA + DHA daily) for 3 months. The glomerular filtrations rate (GFR) fell by 18·0 ± 9·6% in the CyA group compared with 8·7 ± 6·8% in the CyA/EPA + DHA group (mean ± SD; $P < 0{\cdot}05$). The effective renal plasma flow (ERPF) fell by 10·6 ± 8·9% in the CyA group and did not change in the CyA/EPA + DHA group ($P < 0{\cdot}05$). The calculated total renal vascular resistance (TRVR) increased by 19·8 ± 14·5% in the CyA group and did not change in the CyA/EPA + DHA group ($P < 0{\cdot}01$). The results of our open pilot study strongly suggest that fish oil can modify CyA-associated renal dysfunction in psoriasis patients.

CASE REPORTS

Primary cutaneous large cell lymphoma of follicular centre cell origin

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History. Male, aged 91 years. For 3 months the patient had rapidly growing tumours on the right lower leg.

Examination. On the medial aspect of the right lower leg there were large, variably infiltrated plaques with an ulcerating tumour above the medial malleolus. There were no palpable lymph nodes.

Laboratory tests. Extensive staging procedures including complete blood cell counts, chest radiography, bone marrow biopsy, computerized abdominal tomography and abdominal echography failed to show extracutaneous involvement.

Histology and immunohistochemistry. Diffuse dermal infiltrates of atypical lymphocytes with irregular vesicular nuclei and one or more nucleoli were seen. The epidermis was not involved and was separated from the infiltrate by a grenz zone. The atypical cells were CD20$^+$, CD22$^+$, HLA-DR$^+$, lambda$^+$, kappa$^-$, and CD3$^-$.

Treatment. Radiotherapy resulted in a complete remission. However, within 2 months there was a relapse on the right upper and lower leg which was initially outside, but afterwards also within the irradiated area. Despite treatment with chlorambucil 10 mg and prednisone 40 mg daily there was further progression of the skin lesions with the development of palpable right inguinal lymph nodes. The patient was then treated with a combination of cyclophosphamide, adriamycin, oncovin, and prednisone (CHOP). Although the skin lesions responded well to this treatment, the general condition of the patient deteriorated and he eventually died, 7 months after diagnosis. An autopsy was not performed.

Comment. Primary cutaneous large cell lymphomas of follicular centre cell origin are not uncommon. These lymphomas generally present with localized skin lesions on the scalp or the trunk. They have an excellent prognosis, when treated either by radiotherapy or with polychemotherapy.[1,2] In elderly patients, this type of lymphoma preferentially presents with localized lesions on the lower legs. The patient presented here illustrates that the prognosis, partly due to their age, is more unfavourable than other patients with this type of cutaneous B-cell lymphoma.

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Histiocytosis X (Langerhans cell histiocytosis)

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History. Female, aged 70 years. The patient had a 2-year history of painful oral soft tissue granulomatous lesions, perianal ulceration, and a papular eruption on the trunk.

Examination. There were numerous brown–red, scaly papules on the back, the sides of the trunk, and under the breasts. A perianal tumour with ulceration was seen. The gingiva and hard palate were hyperplastic with ulceration.

Laboratory tests. A chest X-ray, a CT-scan of the abdomen, and a bone-scan were normal.

Histology. In the dermis there was a band-like infiltrate that was epidermotropic and which consisted mainly of histiocytes and lymphocytes with a few eosinophils. The histiocytic cells stained positive for OKT 6, OK 1A, Heca, Leu 3, LN-3, and S-100 protein.

Treatment. Irradiation of the oral and perianal lesions gave good results but a relapse soon followed. The skin lesions cleared with local applications of mustine hydrochloride but because of a contact allergic reaction this treatment had to be discontinued. Neither prednisone alone nor etoposide (VP 16) alone were successful, but a combination therapy of etoposide 170 mg/day and prednisone 60 mg/day for 4 days every 3 weeks gave a satisfactory improvement.

Comment. Histiocytosis X (HX) in the adult showing the features of Letterer–Siwe disease is very rare. HX occurs predominantly in children and is classified into three clinical forms: Letterer–Siwe disease, Hand–Schuller–Christian disease and eosinophilic granuloma. Transitional stages are known.[1] Various topical and systemic drugs have been with used varying results.[2] In our patient the combination of VP 16 with prednisone was successful.

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Angiosarcoma

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History. Female, aged 63 years. For 1·5 years a lesion had been present on the right lower leg which in the past few months had been rapidly expanding.

Examination. On the right lower leg there was an ill-defined redish-blue plaque with a number of small tumours. There was no enlargement of the regional lymph nodes.

Histology. The dermis showed an area of closely packed, atypical epithelioid cells with pleiomorphic nuclei, and with formation of small vascular spaces. Sporadic mitotic figures were seen.

Immunohistochemistry. Vimentine$^+$, *Ulex europeus*$^+$, Factor VIII$^+$.

Treatment. Amputation of the right lower leg was performed. After a follow-up period of 10 months there were no signs of metastases.

Comment. Angiosarcoma is a rare tumour. Most patients are elderly people who present with lesions on the head and neck. In about 10% of the patients, long lasting lymphoedema or previous skin radiation are aetiological factors. The treatment of choice is excision often in combination with radiotherapy. The prognosis is poor, with only 12% of the patients surviving for more than 5 years.

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The proteus syndrome

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History. Male, aged 2 years. Since birth, the patient has had lesions. His development was normal.

Examination. There were large subcutaneous tumours on both sides of the trunk, also a naevus flammeus. Both feet showed marked disproportion with megalodactylism (Fig. 1). A linear verrucous epidermal naevus was present on the neck.

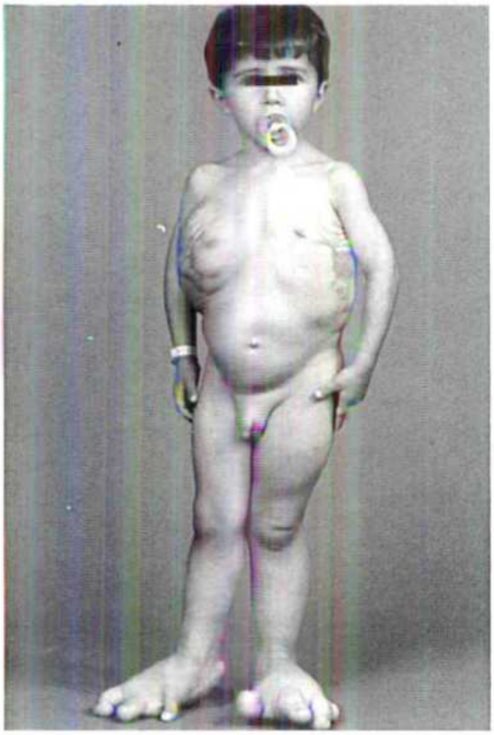

FIGURE 1. Large subcutaneous tumours on the chest with a naevus flammeus and grossly deformed feet.

Laboratory investigations. A CT scan of the skull showed asymmetry and an NMR scan of the chest showed lymphangiomas or haemangiomas.

Comment. The proteus syndrome is a rare congenital hamartomatous disorder that should be differentiated from the Klippel–Trénaunay syndrome and neurofibromatosis. The aetiology is not known, but it is probably due to a mutation of a tissue growth-factor receptor.

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Acanthosis nigricans-like epidermal naevus and Klippel–Trénaunay syndrome

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History. Female, aged 17 years. At the age of 2, epidermal naevi were seen on the neck and then on the face, the scalp and the arms. At age 3, the difference in the length of legs was noticed.

Examination. On both sides of the neck, extending over the cheeks, on the chin, the scalp, the abdomen and the upper side of the arms, were seen almost symmetrical, yellow–brown warty lesions forming wavy bands. The lesions in the neck had a papillomatous appearance (Fig. 1). There was marked hypertrophy with marked varicose veins of the right leg, and a compensatory scoliosis.

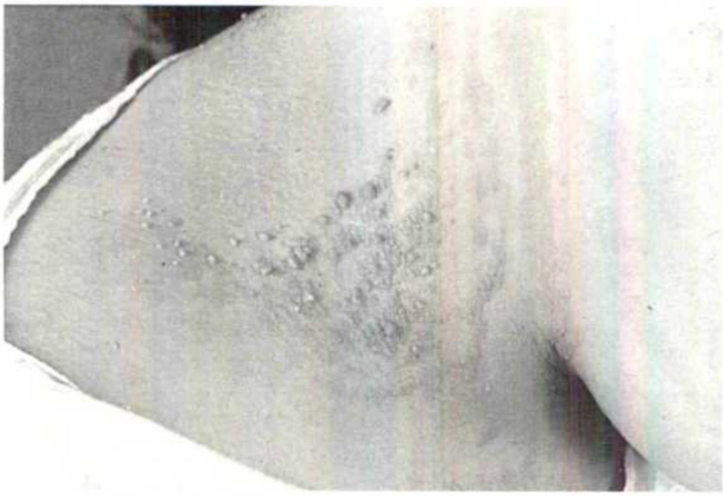

FIGURE 1. Band-like papillomatous and verrucous lesions.

Histology. A skin biopsy from the neck showed hyperkeratosis with marked papillomatosis and a relatively thin epidermis.

Comment. This patient with the combination of an epidermal naevus and hemihypertrophy should not be classified into the Proteus syndrome as she showed a well-proportioned hemihypertrophy. This contrasts to the hypertrophy in many of the previously reported cases with a combination of epidermal naevus and Klippel–Trénaunay syndrome, and which are now considered as examples of the Proteus syndrome.

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Smooth muscle hamartoma

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History and examination. Male, aged 2 years. The back showed a nummular patch slightly

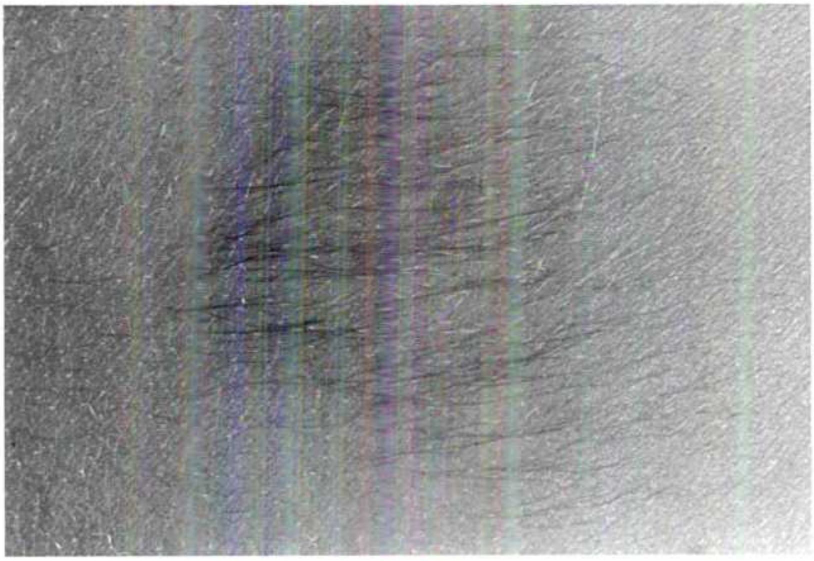

FIGURE 1. Pale-brown area with mild hypertrichosis on the back.

elevated and pale brown in colour with mild hypertrichosis (Fig. 1). This had been present since birth.

Histology. A biopsy showed numerous, thick, well-defined bundles of smooth muscle cells.

Comment. The smooth muscle hamartoma is a rare naevoid condition and is usually present at birth or may develop during childhood. In some cases there is an association with Becker's melanosis. The smooth muscle hamartoma should be differentiated from a piloleiomyoma in which an interlacing network of ill-defined smooth muscle bundles is seen.

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Infantile myofibromatosis (multicentric type)

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History. Female, aged 1 year. Shortly after birth a solitary nodule on the left upper arm was noticed. Impairment of the growth and function of the left leg was also observed. The family history was unremarkable.

Examination. On the left upper arm there was an erythematous nodule and there was a subcutaneous nodule on the flexor surface of the right upper leg (Fig. 1). Three further nodules were present on the left leg and back.

Laboratory tests. Examination of the internal organs and the central nervous system showed

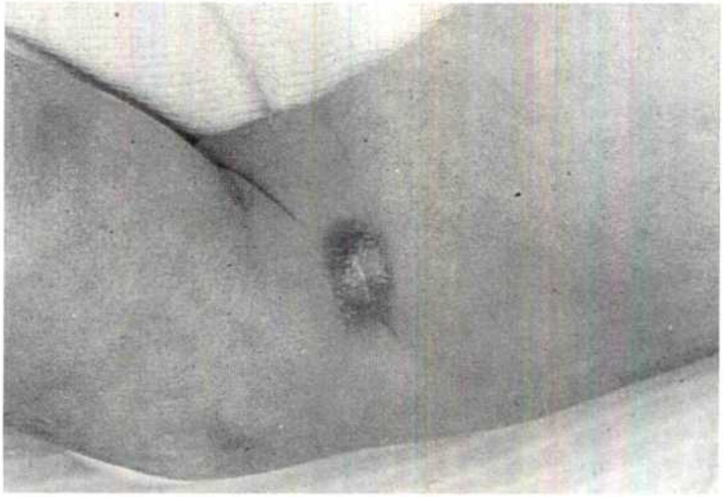

FIGURE 1. Pale red indurated nodule on the arm.

no lesions. Atrophy of the left leg with a flaccid paralysis, due to compression of nerves was noted.

Histology. A biopsy of the nodule on the left arm and left leg showed interwoven bundles consisting of spindle-shaped myofibroblasts.

Comment. Infantile myofibromatosis is a benign tumorous process, probably of hamartomatous origin, which consists of cells that show the characteristics of myofibroblasts. The solitary type is more common (> 50% of the cases) than the multicentric type, and manifests itself as a single nodule in the soft tissues or in the bones. In the multicentric type, multiple lesions are found in the soft tissues, internal organs and bones. The lesions seem to grow chiefly during the immediate perinatal period, but enlargement or formation of new lesions can also be observed in later life. Spontaneous regression of lesions has been described. Solitary tumours in soft tissues and skeleton have an excellent prognosis and in the multicentric type the prognosis depends on the location of the lesions.

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Anetodermia and borreliosis

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History. Female, aged 39 years. For the past year the patient had slowly enlarging lesions on the back, associated with symptoms of fatigue, diarrhoea and arthralgia. Shortly before, the patient had been bitten by ticks in Corsica.

Examination. Many lenticular skin-coloured lesions were seen with a wrinkled appearance and herniation.

Histology. A biopsy showed absence of elastin fibres in the reticular dermis.

Laboratory tests. Borrelia serology 1:160.

Treatment. Tetracycline (1·5 g/day) for 1 month and following treatment, the serology went down to zero. No new skin lesions appeared and the systemic symptoms disappeared.

Comment. Anetodermia can be divided into primary and secondary forms. The condition is due to a loss of elastic fibres. In a number of studies other defects affecting eyes, bone and heart are mentioned besides the skin lesions, suggesting a more generalized disease.[1] It has been suggested that anetoderma and scleroderma can be the consequence of an infection with *Borrelia burgdorferi*.[2] The findings in the present case may support this hypothesis.

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Chrome dermatitis

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History. Male, aged 39 years. The patient had worked as a chrome galvanizer for 6 months, when he developed follicular lesions on the face.

Examination. Multiple dome-shaped erythematous papules with a follicular distribution were present on the brows and around the mouth and nostrils. (Fig. 1).

Laboratory tests. Patch tests with potassium chromate and bichromate 0·5% in water were

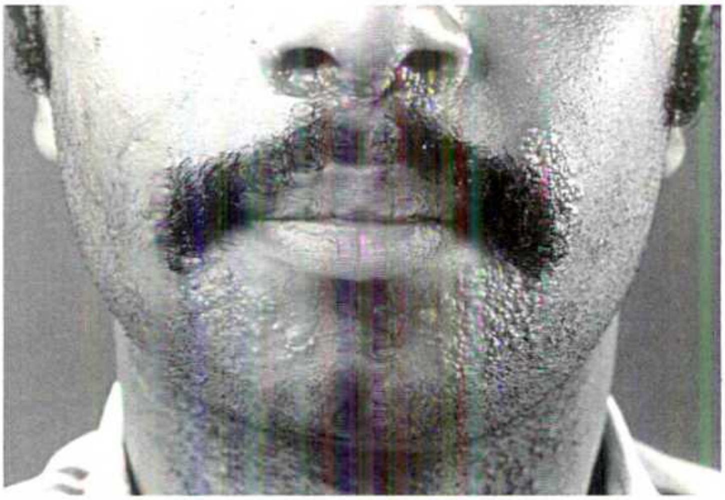

FIGURE 1. Dome-shaped papules around the mouth and in the nostrils.

negative after 48 h, but were positive at 72 h. X-ray examination of the chest and an ENT-examination showed no abnormalities.

Histology. A biopsy showed granulomatous inflammation around the hair follicles and consisted of histiocytes, lymphocytes and polymorphonuclear granulocytes.

Comment. Although the patient wore protective clothing and an ill-fitting mask, his face came into contact with a chrome-containing mist, released by the galvanizing process. Chromates are well known to cause a toxic follicular dermatitis, skin ulceration and even perforation of the nasal mucosa. Because of the positive patch tests for chromate, we conclude that this patient had both a toxic and an allergic chrome dermatitis.

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Multiple eyelid hidrocystoma, eccrine poromatosis, hypodontia, hypotrichosis syndrome ('Schöpf syndrome')

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History. Female, aged 73 years. Since childhood the patient had the abnormalities mentioned below as well as hypodontia with cone-shaped teeth. A year before, she had received treatment for a basal cell carcinoma of the face and carcinoma of the left breast.

Family history. A sister showed similar cutaneous findings.

Examination. Multiple 1–4 mm cysts were seen on the margins of the upper and lower eyelids

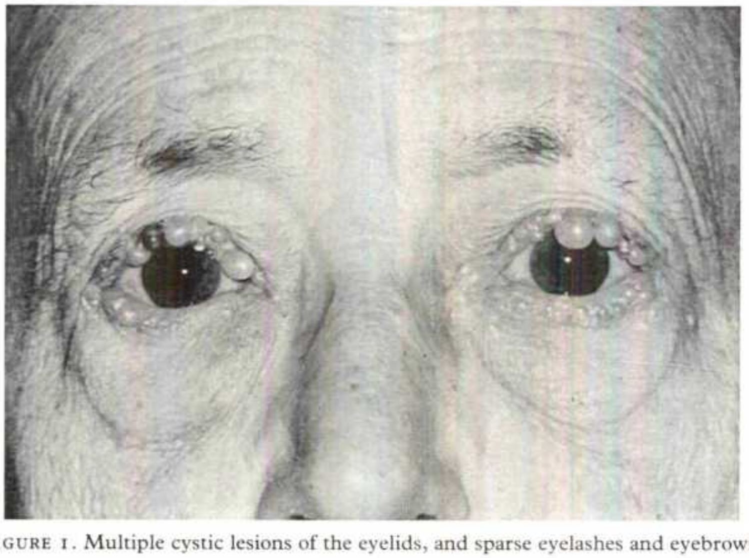

FIGURE 1. Multiple cystic lesions of the eyelids, and sparse eyelashes and eyebrows.

(Fig. 1). The eyelashes and eyebrows were sparse and the scalp hair thin. The palms and soles showed hyperkeratosis with multiple small confluent papules. The nails were hypoplastic.

Histology. A biopsy from the eyelid showed an eccrine hydrocystoma. A biopsy from the palm revealed mild hyperkeratosis and basal projections of thin anastomosing strands of basaloid cells with focal lumen formation, typical of eccrine poromatosis.

Comment. Since the original report by Schöpf *et al.* in 1971, several patients with this rare syndrome have been described. One particular case[1] also showed a squamous cell carcinoma of the palm. The histological findings in our patient are similar to those described by Nordin, Mansson and Svensson,[2] the palmo-plantar lesions being those of eccrine poromatosis.

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Efficacy of intravenous immunoglobulins in sclerodermatomyositis

SIR, The efficacy of intravenous immunoglobulins has recently been reported in a case of severe polymyositis.[1] This case report encouraged us to try this treatment in a young girl suffering from severe sclerodermatomyositis.

A diagnosis of dermatomyositis was initially made in our patient at the age of 12 years. She was successfully treated with prednisolone (1 mg/kg per day). However, a severe relapse occurred after 17 months, while receiving 5 mg of prednisolone per day. Laboratory findings included raised muscle enzymes, creatine kinase: 1675 IU/l (normal 15–120 IU/l); lactate dehydrogenase: 1892 IU/l (normal 140–290 IU/l); aldolase: 25 IU/l (normal 0–7·6 IU/l), a positive homogeneous and nucleolar antinuclear antibody titre of 1/1000, negative Farr's test, the presence of anti-SSA and anti-SSB antibodies, negative anticentromere and anti-Scl 70 antibodies and negative Waaler-Rose latex test, normal total complement and fractions. The chest X-ray was normal. The pulmonary function tests gave a vital capacity of 53% with decreased CO diffusion of 58%.

Treatment with prednisolone at a dose of 2 mg/kg per day for 6 weeks together with 1 mg/day of colchicine for the calcifications produced no improvement. There was sclerodermoid involvement of the face with flexion deformities of the fingers associated with necrosis of the digits. She was no longer able to stand unassisted and was unable to climb. The muscle enzymes remained very elevated and the vital capacity further deteriorated to 33% of normal. She subsequently developed involvement of the middle third of the oesophagus with moderate reflux and adverse effects due to the systemic steroids.

Treatment with i.v. immunoglobulins was started at 400 mg/kg per day for 5 days with repeated courses every 10 days. The systemic steroids were gradually decreased and colchicine was replaced by aluminium hydroxide (600 mg/day).[2] After the first course of treatment, the child was able to stand with support and after the second course, she was able to walk up and down three flights of stairs and ride a bicycle for 10 min. The treatment was continued for 2 months, followed by 2-day courses once a month (1 g/kg per day). After 6 months, the recovery of muscular force was almost complete and the scleroderma-like infiltration improved, the calcification regressed and there was healing of the digital necrotic areas. There was improvement of the lung function tests but the CO diffusion was persistently reduced. The lactate dehydrogenase was 411 IU/l, creatine kinase was 29 IU/l and aldolase was 7·4 IU/l. The immunological results were unchanged. At this time, the dose of prednisolone was only 15 mg/day.

Treatment with i.v. immunoglobulins was very effective in this case and allowed a reduction in the dose of systemic steroids, producing a subjective and objective improvement as regards muscular performance, a lowering of the elevated muscle enzymes and an improvement in the pulmonary function tests. Though dermatomyositis can sometimes spontaneously improve, in our patient the response after the first course of intravenous immunoglobulin treatment was dramatic.

Intravenous immunoglobulins appear to be beneficial in several disorders, including idiopathic thrombocytopenic purpura[3] and myasthenia gravis,[4] but the exact mechanism remains unclear. Intravenous immunoglobulin therapy may be effective in autoimmune disorders by providing a source of anti-idiotypic antibodies. In experimental animal models the production of antibodies declined following treatment with a specific anti-idiotype.[5]

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Commercial assay for steroid sulphatase activity in X-linked ichthyosis

SIR, Recessive X-linked ichthyosis is a metabolic disorder in which there is a deficiency of the enzyme steroid sulphatase.[1] In heterozygotes, enzyme activity is lower than in normal females.[2] Although in typical X-linked ichthyosis the scales tend to be larger and darker and affect more of the skin surface of the body than in the autosomal dominant type of ichthyosis, the distinction between the two types is often difficult. Determination of steroid sulphatase activity has made it possible to distinguish clearly the two types from each other.[3] Also it has been possible to detect individuals who are heterozygous for X-linked ichthyosis so that genetic counselling is possible.[4] Despite the value of the measurement of steroid sulphatase there are up to now only a few centres which carry out this estimation. We have developed a commercial assay for the steroid sulphatase activity of peripheral blood lymphocytes.[4] A radioassay using enzymatic desulphation requires 10 ml of whole blood to which is added EDTA or CPD for anticoagulation. The prevalence of X-linked ichthyosis has been estimated at 1 in 6000 of the population,[5] and that of the autosomal dominant type of ichthyosis vulgaris at between 1 in 250 and 1 in 320.[5] We hope that this new assay will allow the measurement of steroid sulphatase on a much larger scale.

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Sunbed pseudoporphyria induced by nalidixic acid

SIR, There have been several recent reports[1–4] of blistering attributed to the prolonged use of UVA sunbeds. In one,[3] the relevance of the various drugs taken by the patients was questioned. We have recently seen a patient in whom we consider drug ingestion to have been of prime importance in the development of the blistering skin lesions.

A 43-year-old female presented in April 1989 with painful blistering of her lower legs. She had been a regular user of a UVA sunbed over a period of 4 months, having three 30 min sessions each week on her front and back. Three weeks prior to the onset of the eruption she had a 6-day course of nalidixic acid, 4 g/day for a urinary tract infection. She had used the sunbed only once while on the drug but stopped on the advice of her general practitioner and recommenced 1 week after completion of therapy. She was not on any other drugs. The onset of blistering was just over 1 week and four sunbed sessions later.

On examination there were bullae and erosions on her shins with a faint background erythema over her lower limbs and trunk. A skin biopsy showed a subepidermal bulla with almost no cellular infiltrate. PAS-positive material was seen in the dermal blood vessels. Direct immunofluorescence (University Department of Dermatology, Glasgow) was negative. Blood urea and porphyrin analysis of blood, urine and stool (Western Infirmary, Glasgow) were normal. The blistering began to improve within 2 weeks of discontinuing use of the sunbed and the lesions healed with scarring and milia formation. She still has some skin fragility and a patchy erythema on her lower legs.

The clinical morphology and the cumulative sunbed exposure in this case are similar to those reported as sunbed blistering or pseudoporphyria. However, the distribution of the blistering was rather atypical, although this pattern has been documented in cases attributed to nalidixic acid photosensitivity.[5] Nalidixic acid is well-documented as a cause of pseudoporphyria and is not unique in producing photosensitivity of this type, which may last for some time after stopping the drug.[6] A number of other drugs are known to cause pseudoporphyria[7,8] and several of the reported cases attributed to sunbeds were noted to have taken potential photosensitizers: frusemide,[3] the oral contraceptive[3,4] and ibuprofen.[1]

While acknowledging that sunbed-induced pseudoporphyria undoubtedly occurs, we suggest that the role of ingested photosensitizers should be considered, even when taken some time before the eruption. In cases of pseudoporphyria where sunbed exposure is a feature, a careful drug history is very important.

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Erosive pustular dermatosis of the leg—a definition

SIR, We enjoyed the letter on 'Erosive pustular dermatosis of the scalp—a definition',[1] but we are sorry that Burton and his colleagues think we have scalped their clinical entity. This was never our intention.

One of the reasons for presenting our patients was to try and cast a little light on the pathogenesis of erosive pustular dermatosis whatever its site. It is our experience that erosive pustular dermatosis of the scalp in the U.K. almost always occurs on previously damaged and clinically atrophic skin. Common forms of preceding damage to the scalp include excessive ultraviolet light, shingles, or even physical trauma sufficient to produce scarring. Exactly the same situation holds good for the legs, so it is not surprising that dermatoliposclerosis was quite often present on our patients. Another feature in common between scalp and shin is that the skin in both these areas is fairly tightly applied to underlying bone.

We quite agree that our patients with erosive pustular dermatosis of the leg are not cases of erosive pustular dermatosis of the scalp, but nevertheless they do show the changes of erosive pustular dermatosis clinically. However, we concede that a scalp, even to a Northerner, looks quite different to a leg. In the meantime, we suggest that erosive pustular dermatosis of the leg should be defined as follows: chronic erosive pustulation confined to the legs of patients with a long-standing history of gravitational ulceration, dermatoliposclerosis and associated skin atrophy. No currently recognized cause of pustulation is present and the histology is non-specific.

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Expression of the myelomonocytic antigens L1 and CD36 in human epidermis

SIR, We read with interest the paper by Kirkham *et al.*[1] detailing the epidermal distribution of the myelomonocyte L1 antigen in inflamed skin as assessed by immunohistochemical staining with the monoclonal antibody Mac 387. Their results were essentially identical to those previously published.[2,3] Although L1 antigen is cytoplasmic, we were struck by the close similarity between its pattern of expression in keratinocytes and the distribution of CD36 (OKM5), a surface antigen, in inflammatory skin diseases such as psoriasis, allergic contact dermatitis and lichen planus as we have reported.[4] In both cases, keratinocyte expression was mainly suprabasal and confined to those areas immediately overlying the dermal inflammatory infiltrate. Keratinocyte CD36 expression has been reported to be induced *in vitro* by interferon-gamma (IFN-γ),[5] a product of activated T lymphocytes, and we have suggested that this finding provided further evidence that cytokine-activated keratinocytes participated in skin immune responses. Kirkham *et al.*[1] arrived at similar conclusions based on their results for L1 antigen expression.

More recently, however, we have explored the distribution of keratinocyte CD36 expression immunohistochemically in tumours of epidermal origin (unpublished data) and found striking but consistent variations in the patterns of expression. In well-differentiated squamous cell carcinoma (SCC) the neoplastic cells, together with adjacent normal keratinocytes expressed CD36, while in poorly differentiated SCC, CD36 expression was absent from the tumour cells. In basal cell carcinoma (BCC), CD36 expression by the tumour cells was absent while strong expression by normal keratinocytes at the shoulders of the lesions was consistently observed in a suprabasal pattern, even in the absence of an inflammatory infiltrate. Subsequently, we examined epidermal tumours for expression of L1 antigen, using Mac 387 antibody and found an identical pattern of expression as for CD36, albeit cytoplasmic, including uninvolved keratinocytes adjacent to SCC and BCC in the absence of an inflammatory infiltrate. In this regard, our results are consistent with those of Gabrielsen *et al.*[6]

We have also attempted to induce keratinocyte CD36 and L1 antigen expression *in vitro* by incubating cultured, normal human keratinocytes with a range of cytokines including IFN-γ, tumour necrosis factor-α and interleukin 1. In all experiments, expression of these antigens could not be induced, despite strong expression of ICAM-1 and HLA-DR.[7] *In vivo*, intradermal administration of IFN-γ[8] failed to induce keratinocyte expression of CD36.

Thus, it appears that rather than being specific for inflammatory cutaneous disease, keratinocyte expression of these two myelomonocytic antigens is widespread in diseases involving the epidermis even in the absence of a conspicuous mononuclear cell infiltrate. Furthermore, since expression is confined to suprabasal keratinocytes in areas of epidermal damage and is unrelated to the underlying inflammatory infiltrate, we suggest that L1 and CD36 expression, rather than being directly associated with cytokines produced by infiltrating mononuclear cells in the papillary dermis, may represent a direct response of keratinocytes, depending on their state of differentiation, to an assortment of environmental cues (injurious stimuli). In support of this hypothesis, keratinocytes respond *in vitro* to a variety of low molecular weight substances including phorbol esters, retinoids and urushiol (the catechol responsible for poison ivy dermatitis) to produce a range of pro-inflammatory cytokines including tumour necrosis factor-α and interleukin 8. This hypothesis would explain the absence of L1 expression in normal skin, but positive L1 staining of normal-appearing oral mucosa where repeated trauma probably exists.[9]

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Topical capsaicin for psoriasis

SIR, The nervous system appears to affect psoriasis through peptides such as substance P (SP) which is released from cutaneous sensory nerve endings.[1] SP-immunoreactive nerve fibres are reported to be increased in the lesional skin in psoriasis,[1,2] and SP binds to receptors on mast cells, inducing degranulation.[1] In a developing lesion, mast cell degranulation is one of the early histological changes,[3] and the release of inflammatory mediators may enhance epidermal and endothelial cell proliferation.[3] SP induces local axon reflex vasodilatation though release of histamine from mast cells and released histamine stimulates other sensory neurones.[1] SP can also increase inflammation through mediators released from macrophages and T cells. SP enhances the production of the keratinocyte-derived inflammatory mediators, interleukin 1 and granulocyte-macrophage colony-stimulating factor which are involved in the pathogenesis of psoriasis.[4]

Capsaicin is derived from plants of the Solanaceae family and depletes SP through inducing degeneration of primary sensory neurones. We treated 10 patients with psoriasis vulgaris (seven men, three women; mean age 32 years) with topical capsaicin in an 8-week open trial. The patients had severe psoriasis with involvement of 15–30% of the total body surface area. The patients were instructed not to take any medication 4 weeks before the treatment and during the trial. The patients were provided with tubes of 0·025% capsaicin in a cream base (GenDerm Corp., Northbrook, IL, U.S.A.). The cream was applied to the psoriatic plaques on one side of the body, four times daily. Psoriatic lesions on the other side were treated with a petrolatum placebo. Evaluation was performed by the same physician before therapy, and then at weekly intervals. At each visit the itching, scaling and erythema were graded according to a four point scale (0, absent; 1, trace; 2, mild; 3, moderate; 4, severe). The severity score was obtained by adding the scores. All of the patients completed the trial. At the end of the study, seven patients showed marked improvement with capsaicin as regards itching, scaling and erythema. In the non-responders, the itching disappeared. There was no significant change in the placebo-treated lesions. The patients tolerated the treatment well, and no side-effects were observed.

The results of this preliminary study show that capsaicin therapy seems to be effective in the treatment of psoriasis, especially in relieving itching, by inhibiting the release of SP from cutaneous sensory neurones.

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Treatment of lichen planus with a short course of oral prednisolone

SIR, A variety of systemic treatments have been suggested for lichen planus but corticosteroids remain the most widely used. There have been no clinical studies of the effectiveness of this therapy although a course of up to 6 weeks is recommended.[1] It is also stated that corticosteroid therapy does not affect the total duration of the disease.[2] We therefore undertook a double-blind study of oral prednisolone in 38 patients with lichen planus. The short duration of the course of steroids allowed the dose not to be reduced gradually. The diagnosis of lichen planus was made on clinical grounds and contraindications to the study were a history of peptic ulceration, diabetes and previous treatment with corticosteroids.

The patients were randomly allocated to either active or placebo therapy coded by the pharmacist. Active treatment consisted of prednisolone 30 mg daily for 10 days and placebo treatment consisted of a similar course of inactive tablets of identical appearance. Hydrocortisone-17-butyrate cream was given for topical treatment with instructions to use it twice daily. Patients were seen on the initial visit prior to entering the study, 6 weeks later then at intervals of 3 months until either the condition had cleared or 2 years had elapsed from the time of the first visit. At each visit the observer recorded a score of severity of the lichen planus on a 10 cm linear analogue scale on which 0 represented 'much less severe' and 10 'very much worse'. A similar assessment was carried out by the patients themselves and there was a separate assessment of degree of itch also on a 10 cm visual analogue. The observer noted any change in the lichen planus according to the following scale: 0, worse; 1, no change; 2, better; 3, clearing; 4, cleared. The Wilcoxon rank sum test was used for statistical analyses. Twenty-eight of the 38 patients completed the study, 14 in the active group and 14 in the placebo group. The profile of both groups in terms of age, duration and severity of lichen planus was very similar.

The time taken for lichen planus to clear was significantly different between the two groups ($P = 0{\cdot}02$). The median time in the prednisolone group was 18 weeks (range 4–61) and in the placebo group was 29 weeks (6–104). Three patients in the placebo group but none in the active group failed to clear within 2 years. At the 6-week visit the linear analogue scores of severity of rash by the patients were significantly different between active and placebo groups ($P = 0{\cdot}01$) (Table 1). The improvement in the observers' linear analogue scores for severity from entry to 6 weeks (score at entry—score at 6 weeks) was significantly different between the two groups ($P < 0{\cdot}05$). The patients' linear analogue scores for itch at 6 weeks failed to show a significant difference, although the median value in the prednisolone group (22) was less than that in the placebo group (30). The observers' scores for change at 6 weeks were significantly different between active and placebo groups ($P < 0{\cdot}05$). At 6 weeks four patients in the prednisolone group were considered cleared and four improved compared with one patient that cleared and one with clearing in the placebo group. At 18 weeks 10 patients in the prednisolone group had cleared compared with seven on placebo.

The side-effects were minimal. One patient experienced mild heartburn while on prednisolone but did not stop treatment and another patient experienced euphoria. There were no side-effects in the placebo group. Three of the 14 patients on prednisolone had a degree of rebound after an initial symptomatic improvement whilst one patient in the placebo group also described a dramatic improvement with a slight relapse after finishing the tablets. Two patients with very severe lichen planus improved on prednisolone, but relapsed so severely that a more prolonged course of corticosteroid therapy was necessary and they were therefore excluded from the study.

TABLE 1. Severity of lichen planus, scored by patient at 6 weeks and improvement of lichen planus, scored by observer at 6 weeks (score at entry minus score at 6 weeks). Median values, range in parentheses

	Prednisolone	Placebo	
Severity (patient)	1·3 (0·4–5·2)	4·9 (1·0–7·1)	$P = 0{\cdot}01$
Improvement (observer)	2·5 (−0·5–7·4)	0·85 (−2·8–5·0)	$P < 0{\cdot}05$

Arndt[1] suggested that corticosteroid therapy is more likely to alter the total duration of disease if given early when the condition is flaring. This was not borne out by our results, although patients entering the study early in the course of their disease cleared slightly sooner than those presenting later. This trend was apparent also in the control group. The total duration of lichen planus in our control group (median 71 weeks) was longer than that in earlier studies in which the medial duration was approximately 8 months.

In conclusion, a short course of oral prednisolone in a moderately high dose and without gradual reduction of the dose is an effective and safe treatment of mild or moderately severe lichen planus.

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British Journal of Dermatology (1990) **123**, 553–554.

Book Reviews

Onychomycoses: Local Antimycotic Treatment. Edited by S.Nolting and H.C.Korting (1990). Berlin Springer-Verlag. Pp. 113. Illustrations: 33 ISBN 3-540-52132-1.

In this pocket-sized volume there is a wealth of information regarding fungi and nail disease. Even though the contents are as a direct result of a conference centred around the use of a bifonazole (Mycospor)/urea paste in the treatment of onychomycosis, the book is a well-balanced account of the epidemiology and pathology of the onychomycoses, various approaches to specific systemic and topical treatment, and the use of a number of keratinolytic agents as alternatives to antifungal therapy. Of the 11 chapters, four are devoted to reports of the German experience with bifonazole/urea and the remainder cover aspects of nail anatomy, physiology, the mycology of onychomycoses, the various approaches used in assessing the extent of onychomycosis before and after treatment, and problems of compliance in the treatment of fungal nail disease. Each chapter is adequately referenced. Although there are few photographic illustrations those presented are of good quality. For such a slim book there is a well structured index, and this is a useful reference book for those interested in fungal nail disease and seeking an alternative method to systemic therapy. Furthermore, this book is a useful contribution in that it brings together much of the published literature on onychomycosis and will provide an excellent source of information for clinicians and mycologists.

M.D.Richardson

Recent Advances in Dermatology—No. 8 Edited by R.H.Champion and R.J.Pye (1990). Edinburgh: Churchill Livingstone. ISBN No 0-443-04168-7. Price: £29.95.

There must be few specialists who are not interested in recent advances pertaining to their field. Sometimes, however, such texts become bogged down in minutiae which seem to have little relevance to the practising clinician. This book is aimed at the practising clinical dermatologist and scores a bull's eye. There are 15 chapters written by different authors, some dermatologists, other being scientists or clinicians from other branches of medicine, but all with experience or expertise in their field.

Some chapters deal with intensely practical subjects such as the management of difficult psoriasis, intensive care in dermatology, porphyria cutanea tarda, etc. These are written in an authoritative but helpful manner. Other chapters deal with some updates in autoimmunity, vascular interventional radiology etc. These chapters contain a section which gives rapid elevation from basic and historical knowledge to a relatively recent plateau of current knowledge which is then critically assessed. At the end of most of the chapters there is a short section tying this into current clinical practice. It would be very difficult to obtain all the information contained in these chapters by general reading. The editors have to be complimented on the uniformity of the writing style, the lucidity and the relative brevity of each chapter. There is a wealth of references in each for anyone who wishes to take the subject further. Inevitably one could argue with one or two statements but these are individual and would be churlish to mention. This is a book which every dermatologist should read and re-read.

J.Thompson

The Ichthyoses: A Guide to Clinical Diagnosis, Genetic Counselling and Therapy. H.Traupe. (1989). Pp. xv + 253; 81 figs. Berlin, Heidelberg: Springer-Verlag. ISBN 0-387-19222-0. Price £58·00.

This is a delightful book which brings together the historical, clinical, pathological and genetic aspects of the ichthyoses, of which there are more than 20 known types. It is primarily aimed at physicians caring for patients with ichthyosis. It also outlines the latest developments in the fields of lipid chemistry, enzymology and molecular genetics and will therefore be of interest to researchers involved in skin biology of human genetics.

In Chapter 1 the author proposes a new and practicable classification of the ichthyoses. There are also very useful tables on clinical, histological and genetic aspects of differential diagnosis. The importance of epidermal lipids and the biochemistry of keratinization is considered by Dr B. Melnick, Dusseldorf. Lipid abnormalities important in the pathogenesis of diseases including X-linked recessive ichthyosis, multiple sulphatase deficiency and Refsum's Syndrome, are described. The analysis of epidermal lipids has proved a useful diagnostic tool for the classification of the lamellar ichthyoses and, in future, may help to provide new therapeutic agents.

The distinct types of ichthyosis are considered in Chapters 2–5, according to the new classification: isolated vulgar ichthyoses e.g. autosomal dominant ichthyosis; associated vulgar ichthyoses, e.g. Refsum's syndrome; isolated congenital ichthyoses, e.g. lamellar ichthyosis; associated congenital ichthyoses e.g. Sjogren-Larsson syndrome. In Chapter 6, recently recognized forms of ichthyosis such as Dorfman's syndrome are described. The mainstay of therapy for most patients is topical, and this is described in detail in Chapter 7 with very useful personal tips. The potential systemic toxicity of topical salicylic acid preparations, especially where the epidermal barrier function is deficient, and the pitfalls of topical therapy in children owing to their greater surface area/weight ratio, are emphasized. Systemic retinoid treatment, including very useful guidelines for patient selection and monitoring, conclude the treatment chapter.

The material in this book is presented clearly and concisely. There is a comprehensive reference list after each sub-section, making for easy access, and the book is well indexed. The clinical illustrations, in black and white, are of high quality, and clearly demonstrate the important features of each disease. The photomicrographs are also very good.

The strongest quality of the book is that the author has considerable experience in both clinical management of patients and research in the field of ichthyosis. I would highly recommend it to those involved in clinical and experimental dermatology and genetics. In the foreward, Rudolf Happle anticipates that the book will be considered a milestone in the literature of keratinizing disorders. I wholeheartedly agree.

D. TILLMAN

British Journal of Dermatology (1990) **123,** 555.

News and Notices

18th World Congress of Dermatology
12-18 June 1992, New York, U.S.A.

The 18th World Congress of Dermatology Organizing Committee and the International League of Dermatological Studies invite the submission of abstracts for short communications to be presented at the 18th Congress. Selected abstracts will be presented in three forms:

Case presentations. Four-minute presentations of clinical cases of exceptional scientific and/or educational interest.

Contributions to clinical and experimental dermatology. Ten-minute presentations of original contributions to clinical or therapeutic investigations, or unusual dermatoses of teaching value to world dermatology.

Poster presentations. Original contributions to clinical and laboratory investigation which can be effectively displayed by illustrative material (graphs, charts and tables). Authors are to be present during specified times for discussion of the posted material.

Abstracts must be submitted on the official Congress Abstract Reproduction Form. Forms and submission guidelines will be available in November 1990 on request from the Secretariat, 18th World Congress of Dermatology, 22 Euclid Street, Woodbury, NJ 08096, U.S.A.

Dermatology Self Assessment Workshop
Guy's Hospital, London, 19 April 1991

This course is designed for both pathologists and dermatologists and should be of interest to registrars studying for postgraduate qualifications as well as established consultants. New material is studied every year. The registration fee of £45 covers the working sessions, lunch and coffee breaks. For further information and registration forms please contact: Miss Linda Lewis, Guy's Hospital, London SE1 9RT, telephone no. 071-955-4584.

RoC European Award in Photodermatology

An award of 50,000 French francs is to be granted to a national of one of the European community nations for a scientific work in the field of photodermatology. Only work undertaken after 1 January 1988 will be considered for the RoC award which will be made in March 1991. Submissions written either in English or French of no more than 15 typed pages should be forwarded to RoC s.a. Medical Department, 50 rue de Seine, 92703 Colombes cedex, France by 15 November 1990.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

Manuscripts. Three copies of all material for publication should be sent to the Editor, Professor S. S. Bleehen, Dermatology Department, Royal Hallamshire Hospital, Sheffield S10 2JF. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be *unmounted* glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these should be grouped on a separate sheet of paper.

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British Journal of Dermatology, Volume 123, Number 4, October 1990

CONTENTS

PAGE

Autoantibodies to nucleolar antigens in systemic scleroderma: clinical correlations
MARIA BŁASZCZYK, MARIA JARZABEK-CHORZELSKA, STEFANIA JABŁOŃSKA, T.CHORZELSKI, ZOFIA KOLACIŃSKA-STRASZ, E.H.BEUTNER AND V.KUMAR 421

Endemic pemphigus foliaceus in western Parana, Brazil (1976–1988)
J.C.EMPINOTTI, L.A.DIAZ, C.R.MARTINS, E.A.RIVITTI, S.A.P.SAMPAIO, C.LOMBARDI, J.A.SANCHES AND COOPERATIVE GROUP FOR FOGO SELVAGEM RESEARCH 431

The association of bullous pemphigoid and malignant disease: a case control study
V.A.VENNING AND F.WOJNAROWSKA 439

Linear IgA disease of adults: association with lymphoproliferative malignancy and possible role of other triggering factors
K.GODFREY, FENELLA WOJNAROWSKA AND J.LEONARD 447

Chronic urticaria and cancer: an epidemiological study of 1155 patients
B.LINDELÖF, B.SIGURGEIRSSON, C.F.WAHLGREN AND G.EKLUND 453

The effects of an abrasive agent on normal skin and on photoaged skin in comparison with topical tretinoin
R.MARKS, S.HILL AND S.P.BARTON 457

Fibroblast–keratinocyte interactions in psoriasis: failure of psoriatic fibroblasts to stimulate keratinocyte proliferation *in vitro*
G.C.PRIESTLEY AND R.LORD 467

Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss
ANASTASIA B.CUA, K-P.WILHELM AND H.I.MAIBACH 473

Determination of terbinafine in nail samples during systemic treatment for onychomycoses
P.J.DYKES, R.THOMAS AND A.Y.FINLAY 481

Epidermal interleukin 1 in normal skin of patients with HIV infection
B.DRENO, B.MILPIED, H.DUTARTRE AND P.LITOUX 487

Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis
R.E.A.WILLIAMS, A.G.GIBSON, T.C.AITCHISON, R.LEVER AND R.M.MACKIE 493

Effect of incisional biopsy on subsequent histology of melanocytic naevi
R.K.CURLEY, M.E.FALLOWFIELD, M.G.COOK AND R.A.MARSDEN 503

Eczematous infiltrated plaques to subcutaneous heparin: a type IV allergic reaction
A.J.BIRCHER, R.FLÜCKIGER AND S.A.BUCHNER 507

Regressing atypical histiocytosis and lymphomatoid papulosis: variants of the same disorder?
R.CERIO AND M.M.BLACK 515

Hereditary woolly hair with ocular involvement
AILEEN E.M.TAYLOR 523

Squamous cell carcinoma complicating hidradenitis suppurativa
A.V.ANSTEY, J.D.WILKINSON AND P.LORD 527

Society Proceedings

Proceedings of the 247th Meeting of the Netherlands Society for Dermatology and Venereology 533

Correspondence

Efficacy of intravenous immunoglobulins in sclerodermatomyositis
C.BODEMER, D.TEILLAC, M.LE BOURGEOIS, B.WECHSLER AND Y.DE PROST 546

Commercial assay for steroid sulphatase activity in X-linked ichthyosis
M.OKANO AND K.YOSHIMOTO 547

Sunbed pseudoporphyria induced by nalidixic acid
D.BILSLAND AND W.S.DOUGLAS 548

Erosive pustular dermatosis of the leg—a definition
J.A.COTTERILL AND S.W.LANIGAN 549

Expression of the myelomonocytic antigens L1 and CD36 in human epidermis
J.N.W.N.BARKER, M.H.ALLEN, C.E.M.GRIFFITHS, B.J.NICKOLOFF AND D.M.MACDONALD 549

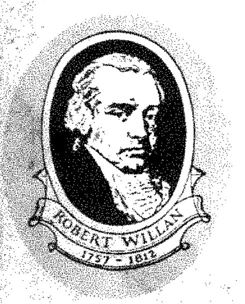
Topical capsaicin for psoriasis
N.KÜRKÇÜOĞLU AND F.ALAYBEYI 550

Treatment of lichen planus with a short course of oral prednisolone
J.K.KELLETT AND R.D.EAD 551

Book Reviews 553

News and Notices 555

Volume [illegible] Number 5 November



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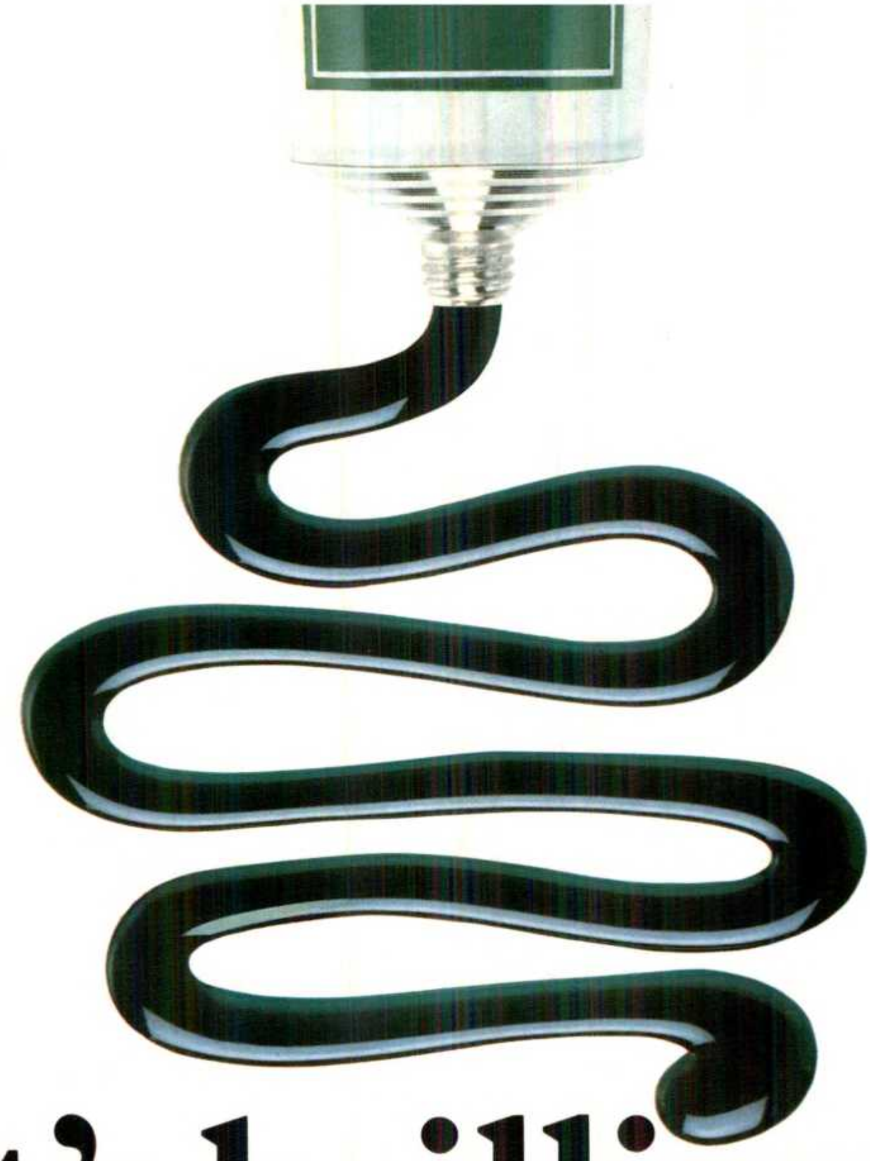

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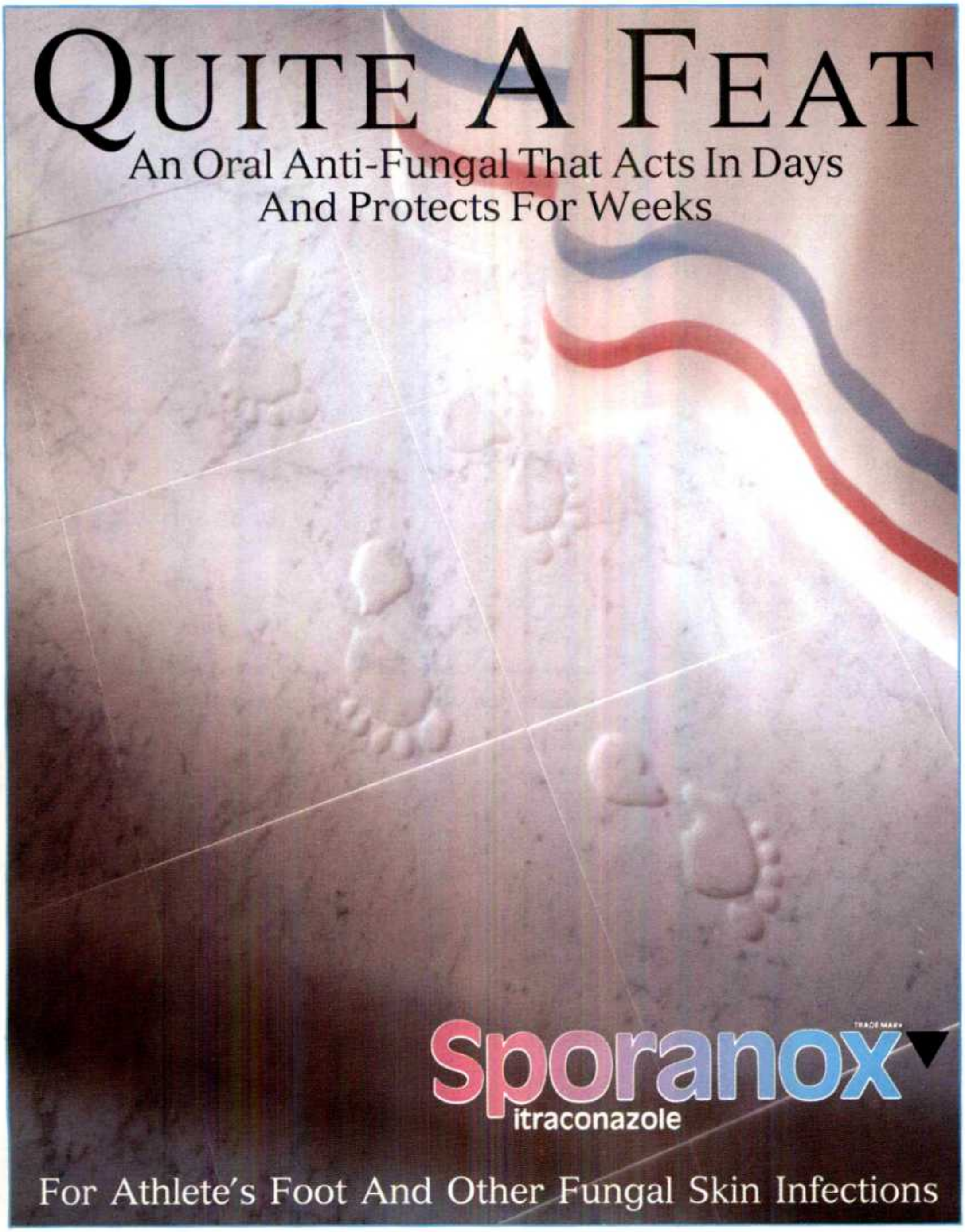
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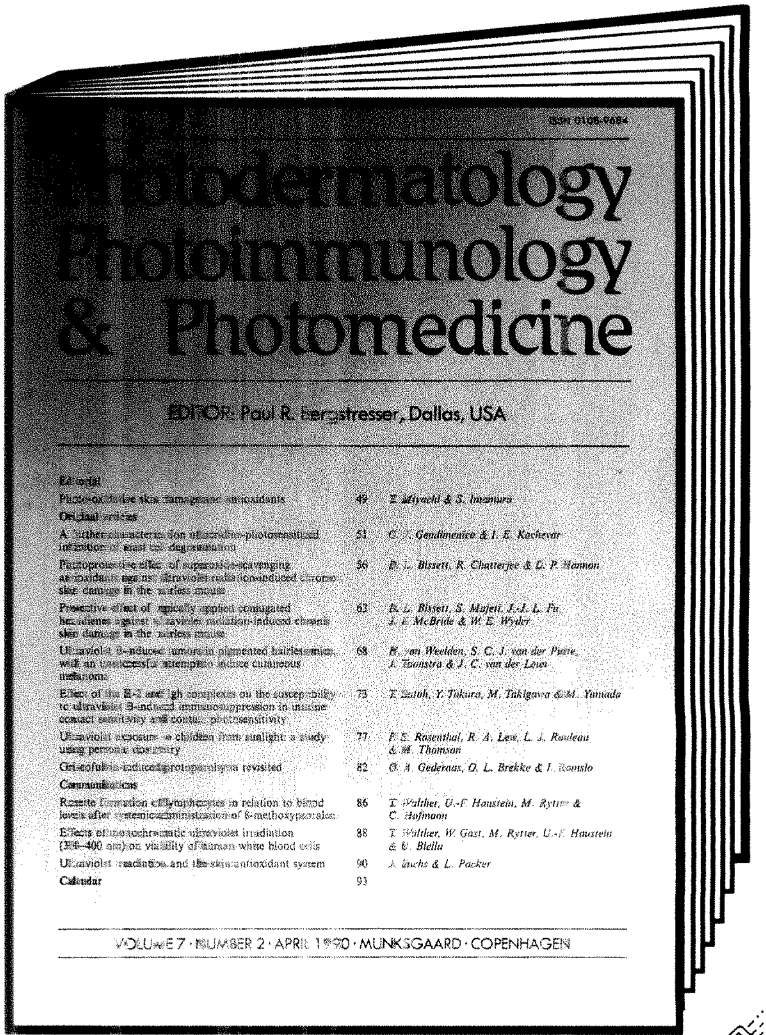

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Photodermatology Photoimmunology & Photomedicine

EDITOR: Paul R. Bergstresser, Dallas, USA

Editorial
Photo-oxidative skin damage and antioxidants 49 Y. Miyachi & S. Imamura
Original articles
A further characterization of acridine-photosensitized inhibition of mast cell degranulation 51 C. J. Gendimenico & I. E. Kochevar
Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse 56 D. L. Bissett, R. Chatterjee & D. P. Hannon
Protective effect of topically applied conjugated hexadienes against ultraviolet radiation-induced chronic skin damage in the hairless mouse 63 D. L. Bissett, S. Majeti, J.-J. L. Fu, J. F. McBride & W. E. Wyder
Ultraviolet B-induced tumors in pigmented hairless mice, with an unsuccessful attempt to induce cutaneous melanoma 68 H. van Weelden, S. C. J. van der Putte, J. Toonstra & J. C. van der Leun
Effect of the H-2 and Igh complexes on the susceptibility to ultraviolet B-induced immunosuppression in murine contact sensitivity and contact photosensitivity 73 T. Satoh, Y. Tokura, M. Takigawa & M. Yamada
Ultraviolet exposure of children from sunlight: a study using personal dosimetry 77 F. S. Rosenthal, R. A. Lew, L. J. Rouleau & M. Thomson
Griseofulvin-induced protoporphyria revisited 82 O. A. Gederaas, O. L. Brekke & J. Romslo
Communications
Rosette formation of lymphocytes in relation to blood levels after systemic administration of 8-methoxypsoralen 86 T. Walther, U.-F. Haustein, M. Rytter & C. Hofmann
Effects of monochromatic ultraviolet irradiation (3[illegible]-400 nm) on viability of human white blood cells 88 T. Walther, W. Gast, M. Rytter, U.-F. Haustein & U. Biella
Ultraviolet irradiation and the skin antioxidant system 90 J. Fuchs & L. Packer
Calendar 93

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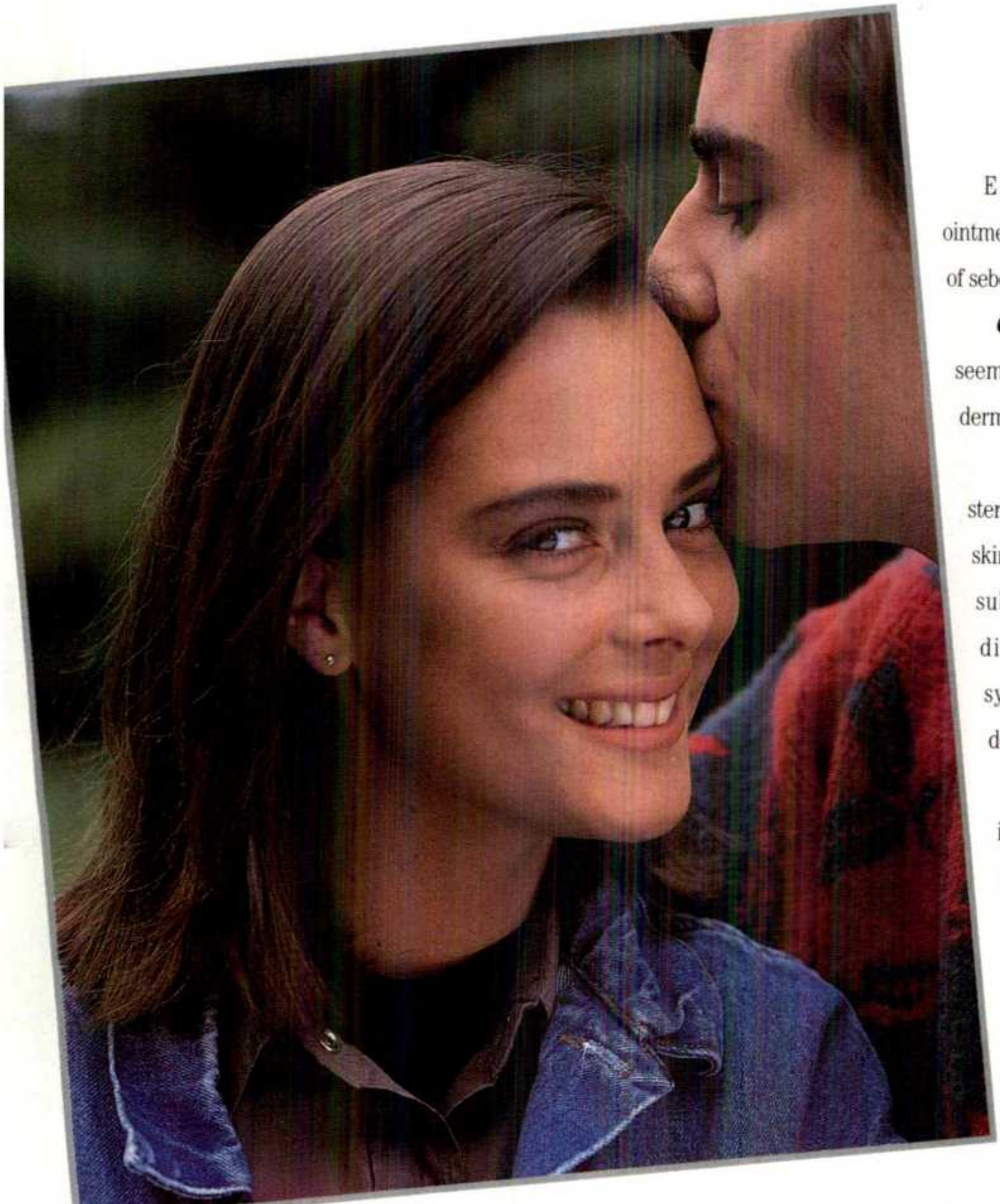

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REFERENCES

1. Boyle J, Burton JL, Faergemann J. Br Med J 1986; 292:28
2. Data on File. Scotia Pharmaceuticals Ltd.

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British Journal of Dermatology (1990) **123**, 557–567.

Immunobiological studies on the alopecic (DEBR) rat

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SUMMARY

The Dundee experimental bald rat (DEBR) has been proposed as an animal model of human alopecia areata, which is suspected of being an autoimmune disease. This study was carried out to establish whether the immunological changes observed in the lesional DEBR rat correlated with studies of human alopecia areata. The immune infiltrate was characterized using immunoperoxidase techniques on cryostat sections of vibrissa follicles. Indirect immunofluorescence was used to quantify the peripheral blood leucocytes. Some parallels were observed in the infiltration of human and DEBR rat follicles by T lymphocytes. In contrast, pre-lesional DEBR rat follicles, which are not available for investigation in human alopecia areata, were not penetrated by leucocytes and MHC class II antigens were expressed in the precortical region of the epidermal component of these follicles. Quantification of peripheral blood leucocytes showed significant increases in both T-lymphocyte subsets during lesional expression. We consider that the pre-lesional form of the rat may provide important information as a model for the pre-lesional and uninvestigated form of alopecia areata in man.

Although the aetiology of alopecia areata in man remains uncertain, an autoimmune mechanism has been the most favoured explanation for the patchy loss of hair.[1] An intense peri-follicular infiltrate of T lymphocytes surrounds and penetrates the follicles, and consists particularly of the helper/inducer subset.[2,3] Perret *et al.*[3] reported the presence of very few B lymphocytes and macrophages and concluded that alopecia areata is a T-lymphocyte-mediated autoimmune disease. The lesion is also associated with the aberrant expression of class I and class II major histocompatibility complex (MHC) antigens.[4-6] Aberrant expression of MHC antigens has been associated with endocrine autoimmunity.[7]

The distribution of T-lymphocyte subsets in the peripheral blood is controversial, with some studies on human alopecia areata suggesting an increase in T-cytotoxic/suppressor cells whilst others suggest an increase in T-helper cells.[8-10] B lymphocytes have received scant attention

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but Kramarczuk–Hordinsky *et al.*[10] reported a reduction in their numbers in the peripheral blood.

In 1985, Wilson described a patchy and progressive loss of hair in a group of germ-free BDIX rats.[11] Due to weaknesses in the strains' ability to breed, the animals were crossed with Wistar rats resulting in the Dundee experimental bald rat (DEBR) which was bred as an animal model for alopecia areata. In their pre-lesional form, the DEBR rats exhibit a perfectly formed pelage until an age of 3·5–4 months when the hypotrichotic lesion becomes progressively expressed over a period of around 4 months and after which the lesion becomes stabilized. Lesional expression is apparent in both vibrissa and pelage hair fibres. The condition of hair loss was suspected of having an underlying immune mechanism after histological examination of skin and vibrissa follicles revealed the presence of a mononuclear cell infiltrate. In the present study, some aspects of the immune system were examined to determine whether the lesion of the DEBR rat paralleled human alopecia areata.

METHODS

Characterization of the cellular infiltrate with monoclonal antibodies in vibrissa follicles

Vibrissa follicles dissected from DEBR and PVG/C (control) rats were snap frozen in liquid nitrogen and 5 μm cryostat sections were cut, dried overnight and fixed in acetone for 20 min, washed in phosphate-buffered saline (PBS), dried and incubated with normal horse serum for 30 min. Removal of the serum was followed by incubation of the sections with the primary antibodies: OX-19 labels T lymphocytes; W3/25 labels T-helper/inducer cells; OX-8 labels T-cytotoxic/suppressor cells; OX-33 labels B lymphocytes; ED-1 labels monocytes/macrophages; P7/7 labels MHC class II antigens. All primary antisera were from Serotec (Bicester, U.K.) and were diluted 1:10. Following primary incubation the sections were washed, incubated with biotinylated anti-mouse serum (Vector Laboratories, Bretton, Peterborough U.K.) for 45 min, washed and incubated with the avidin–biotin complex solution (Vector Laboratories) for 45 min. The reaction was visualized with 3,3 diaminobenzidine and hydrogen peroxide (Sigma, Poole, Dorset, U.K.). The slides were counterstained with Mayer's haematoxylin, dehydrated through an ascending series of ethanol, cleared in Histoclear (National Diagnostics) and mounted in DPX (British Drug Houses Ltd., Glasgow, U.K.). Negative controls using PBS in place of the monoclonal antibodies (MAbs) were provided.

Peripheral blood leucocyte counts

Animals in the following categories of lesional state were used: nine pre-lesional, 12 actively lesional and 10 late lesional DEBR rats. Nine Wistar rats were used as controls.

Blood was obtained from anaesthetized rats by cardiac puncture, heparinized and layered onto a Ficoll/Triosill gradient (Sigma, density 1083 g/ml). After centrifugation at 400 **g** for 40 min, the leucocyte layer was aspirated, washed twice with PBS and counted using a Coulter-counter.

Labelling of leucocytes by indirect immunofluoresence

Following determination of the total cell number, the suspension was diluted to contain 4 × 10^6 cells/ml. For each rat sample, 50 μl of the cell suspension was aliquoted into a column of wells in a 96-well microtitre plate. To each aliquot 50 μl of the relevant, diluted MAb was added, incubated for 30 min on a shaker-bath at room temperature and washed four times at 4°C with a PBS/5% plasma mixture. Cells were subsequently incubated with 50 μl of sheep fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Scottish Antibody Production Unit, Carluke, U.K.; dilution 1:30) for 20 min. The cells were washed four times as previously described and fixed in 2% formaldehyde in PBS and were transferred to microscope slides for counting, using a Leitz Orthoplan fluorescence microscope. Cell counts were expressed as the percentage fluorescing within a total of 100–200 cells. Student's *t*-test was carried out on the data obtained.

RESULTS

Immunohistochemical characterization of inflammation

Control (PVG/C rats) and pre-lesional (DEBR) vibrissa follicles showed similar staining. Few or no lymphocytes were observed within the dermal papilla, dermal sheath, epidermal matrix and sinus area of these follicles. This was consistent for T and B lymphocytes and the monocyte/macrophage MAbs (Fig. 1). However, whereas the control follicles exhibited a negative result when stained for the presence of MHC class II antigens, a positive result was observed within the pre-cortical area of the pre-lesional DEBR follicles (Fig. 2).

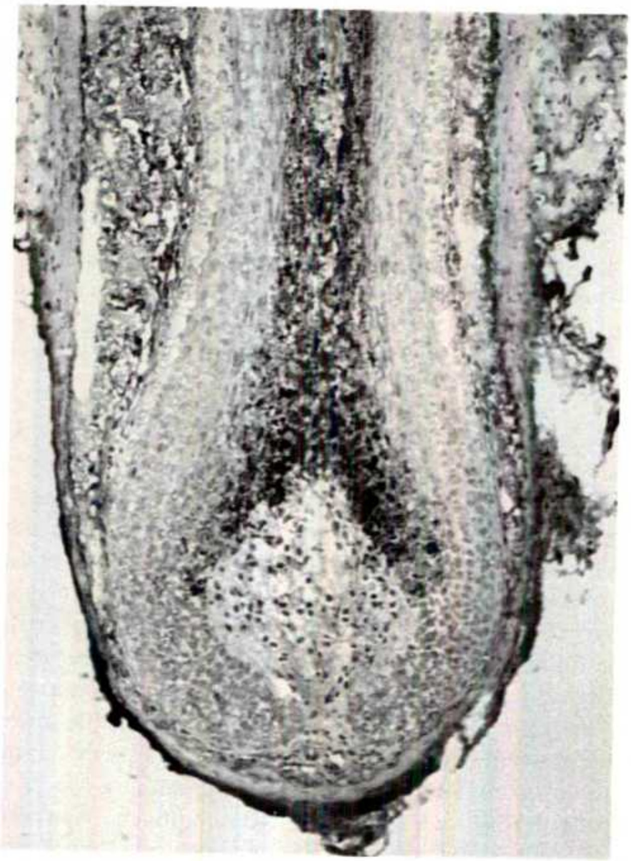

FIGURE 1. Immunoperoxidase labelling of T lymphocytes (OX-19) within a pre-lesional vibrissa follicle. No positive staining has occurred within any of the follicular components (× 170).

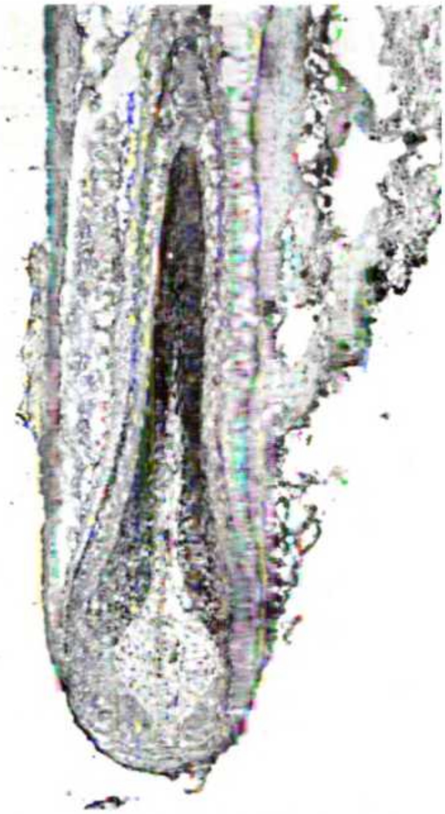

FIGURE 2. Immunoperoxidase labelling of MHC class II antigens within a pre-lesional vibrissa follicle. Positive staining has occurred within the pre-cortical region of the follicular epidermis (× 90).

Mid-lesional follicles revealed the presence of T and B lymphocytes within the papilla and basal stalk area (Fig. 3). There was some evidence of positive staining within the thickened dermal sheath. These cells were also observed within the area of the papillary–epidermal junction. Both T-lymphocyte subsets were expressed within the dermal papilla in an even distribution and within the thickened dermal sheath. Vibrissa follicles labelled with the anti-monocyte/macrophage marker (ED1) revealed a concentration of these cells within the apical region of the dermal papilla, on the basement membrane zone and within the pre-cortical region of the epidermis. The number of these cells was large within these areas but they were also present in significant numbers towards the base of the dermal papilla, and the dermal sheath. Within the epidermis the cells were mainly concentrated within the lower pre-cortical region but some were observed above the bulbs (Fig. 4). These cells were evidently monocytes because the W3/25 antibody marks macrophages and such intense staining was not observed around the upper pole of the papilla. Expression of class II MHC antigens was restricted to a few cells within the dermal papilla and dermal sheath area. Some positive cells were also observed within the area of epidermis immediately adjacent to the apical region of the dermal papilla in mid-lesional follicles.

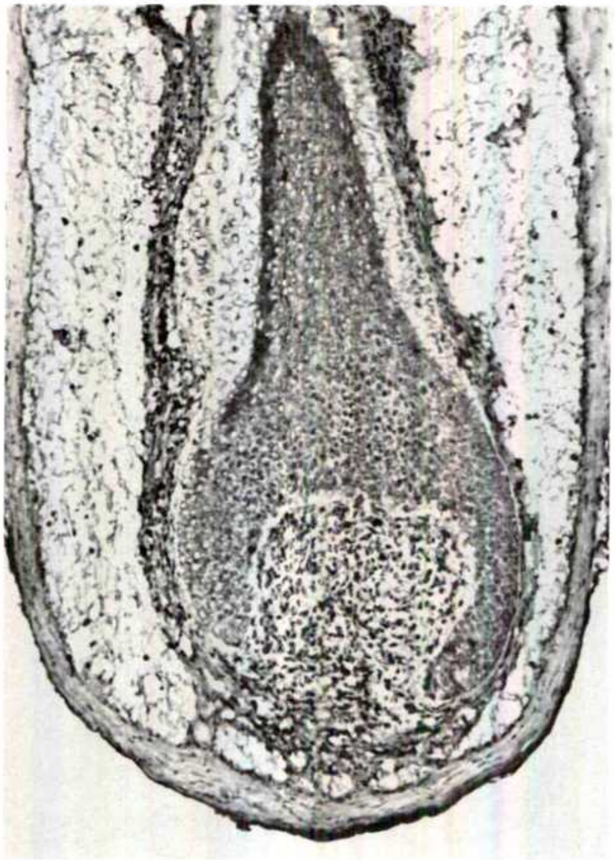

FIGURE 3. Immunoperoxidase labelling of T lymphocytes (OX-19) within a mid-lesional vibrissa follicle. Positive staining is apparent within the sinus area, and dermal papilla of the follicle (× 170).

Quantification of white blood cells within the peripheral blood

The absolute numbers of peripheral white blood cells are presented in Table 1.

Total T lymphocyte counts as quantified by labelling the cells with the MAb OX-19 (Fig. 5) showed a slight but statistically insignificant increase in prelesional DEBR rats when compared with values obtained from control rats. However, rats which actively showed the balding lesion had significantly raised numbers of T lymphocytes compared to controls ($P < 0{\cdot}0001$) and pre-lesional DEBR rats ($P < 0{\cdot}01$). These high T-lymphocyte values persisted when the lesions had stabilized (i.e. late lesional, LL) but at reduced levels of significance when compared with control rats ($P < 0{\cdot}01$). The difference in T-lymphocyte levels was not significant ($P < 0{\cdot}6$) when stable and pre-lesional rats were compared.

Although there was no significant difference in the numbers of cells of the T-helper/inducer subset between pre-lesional and control rats, a large increase in these cells was observed with the onset of the active lesion. Their numbers were significantly different from both control and pre-lesional rats ($P < 0{\cdot}0001$). The T-helper/inducer cells were maintained at a highly significant level compared with control and pre-lesional DEBR rats when the lesion had stabilized in the late lesion ($P < 0{\cdot}0001$) (Fig. 6).

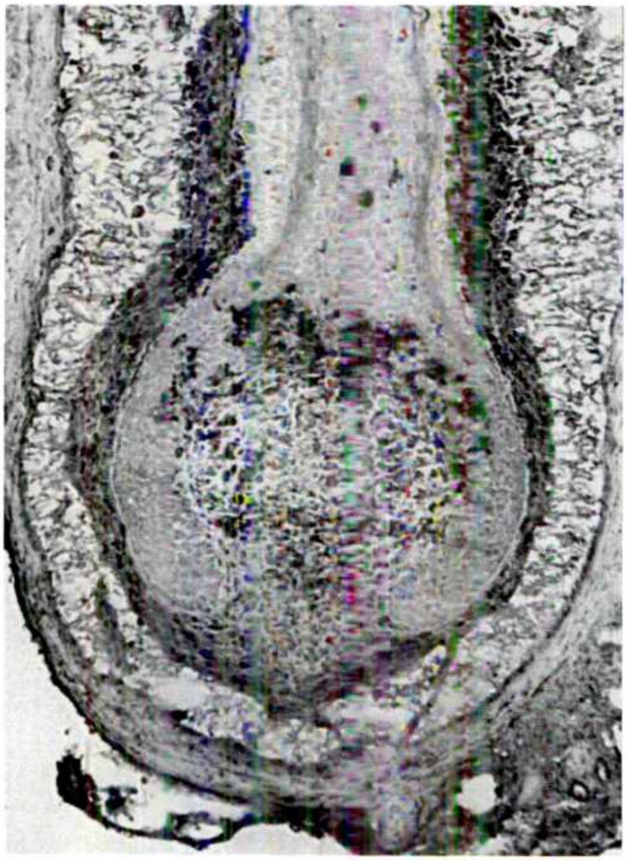

FIGURE 4. Immunoperoxidase labelling of monocytes (ED1) within a mid-lesional vibrissa follicle. Positive staining has occurred within the dermal component and within the epidermal component surrounding the papillary apical dome. Some positive cells are in evidence within the suprabulbar epithelium (× 170).

TABLE 1. Mean variations in absolute numbers of leucocytes with lesional expression. Figures in parentheses represent averages of total numbers of cells counted

Type of rat	T lymphocytes OX-19	T-helper/inducer cells W3/25	T-cytotoxic/suppressor cells OX-8	B lymphocytes OX-33	Monocytes and macrophages ED1
Control	68 ± 10·7 (116·3 ± 20·6)	54·6 ± 26 (132·8 ± 41·2)	41·4 ± 9·1 (121·1 ± 21·5)	13·2 ± 4·8 (109 ± 12)	13·8 ± 4·5 (106·4 ± 11·5)
Pre-lesional	70·7 ± 17·5 (119 ± 20·4)	59·5 ± 19·9 (130·3 ± 56·8)	26·8 ± 13·2 (140·5 ± 37·1)	20·4 ± 8·7 (152 ± 45·0)	8·75 ± 5·6 (138·6 ± 45·1)
Actively lesional	87·8 ± 15·5 (118 ± 16·6)	89·6 ± 20·8 (126·0 ± 24·9)	36·5 ± 15·8 (129·4 ± 41·2)	14·9 ± 9·3 (124 + 19·9)	11·4 ± 8·6 (114 ± 13·2)
Late lesional	82·5 ± 14·5 (121·5 ± 13·9)	82·4 ± 17·5 (119·6 ± 17)	43·4 ± 17·6 (126·1 ± 26·3)	8·4 ± 3·9 126·7 ± 34·9	3·9 ± 2·2 (109 ± 7·5)

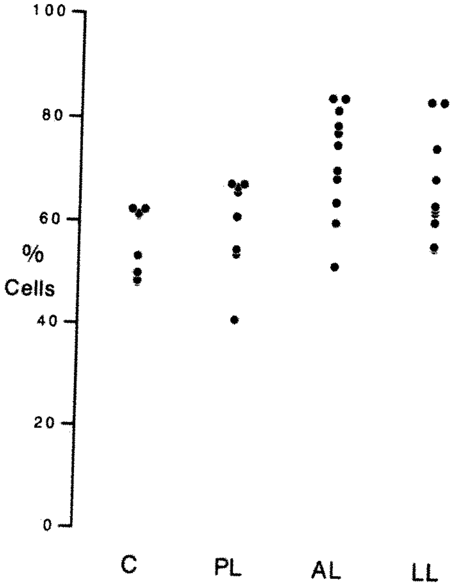

FIGURE 5. Labelling of peripheral T lymphocytes from control (C), pre-lesional (PL), actively lesional (AL) and late lesional (LL) rats. A significant increase in numbers is associated with lesional expression ($0{\cdot}01 < P < 0{\cdot}0001$).

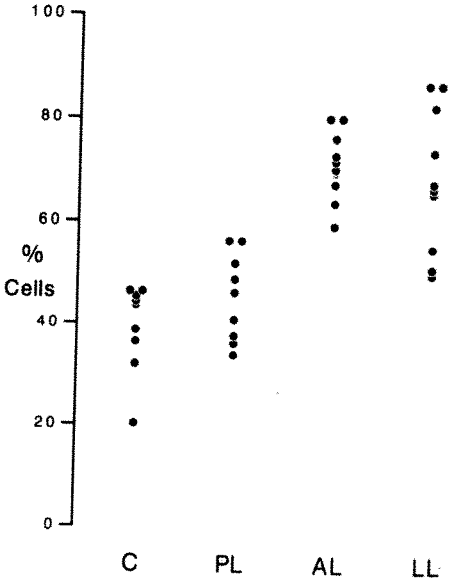

FIGURE 6. Labelling of peripheral T-helper/inducer lymphocytes from control (C), pre-lesional (PL), actively lesional (AL) and late lesional (LL) rats. A significant increase is associated with lesional expression ($P < 0{\cdot}0001$).

DEBR rats within the pre-lesional group had significantly reduced levels of T lymphocytes from the cytotoxic/suppressor subset ($P < 0{\cdot}01$) when compared with control rats. The numbers of these cells increased during lesional expression to a level which was not significantly different from control rats but different from those of pre-lesional rats ($P < 0{\cdot}01$). This elevated level of T-cytotoxic/suppressor cells was maintained throughout the course of the lesion to the later stages (Fig. 7).

There was no difference between the levels of B lymphocytes in pre-lesional rats and control rats. However, a non-significant reduction in the numbers of these cells during the active lesion stage became significant in the late lesion stage when compared with pre-lesional and control rats ($0{\cdot}001 < P < 0{\cdot}01$) (Fig. 8).

Combined levels of monocytes and macrophages were measured by labelling these cells with the antibody ED1 (Fig. 9). Levels within pre-lesional rats were significantly reduced when compared with control rats ($P < 0{\cdot}02$). During the expression of the lesion an elevation in the levels of these cells was observed. Later, when the lesion stabilized, the numbers were reduced to levels well below that of the controls and pre-lesional states ($P < 0{\cdot}0001$).

DISCUSSION

In the DEBR rat follicles the infiltrate contained the two T-lymphocyte subsets in approximately equal numbers. Studies on human alopecia areata have concentrated mainly on the distribution of the T-lymphocyte subpopulations within the vicinity of the follicular end bulbs

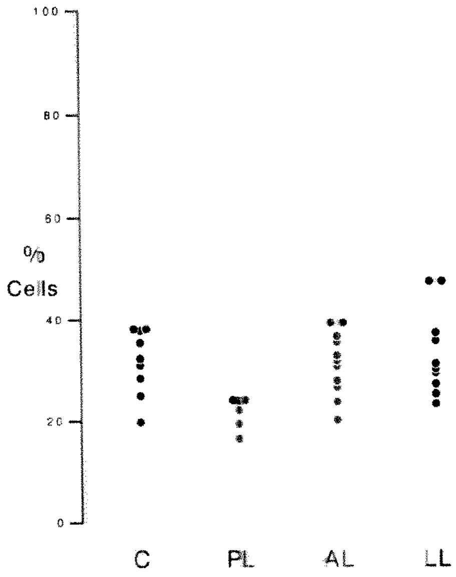

FIGURE 7. Labelling of peripheral T-cytotoxic/suppressor lymphocytes from control (C), pre-lesional (PL), actively lesional (AL) and late lesional (LL) rats. Pre-lesional levels are significantly reduced compared with controls ($P < 0{\cdot}01$). A significant increase over pre-lesional rats is shown during expression of the lesion ($P < 0{\cdot}01$).

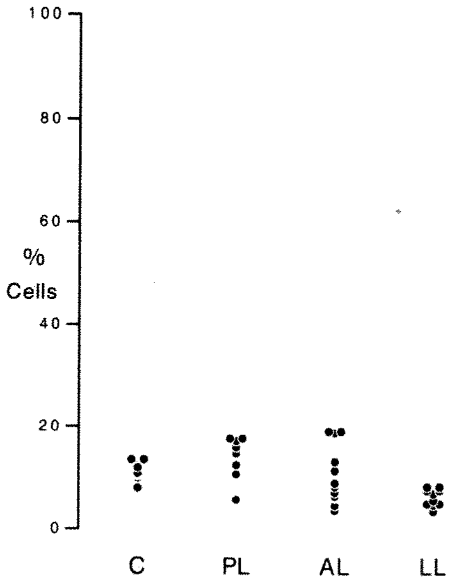

FIGURE 8. Labelling of peripheral B lymphocytes from control (C), pre-lesional (PL), actively lesional (AL) and late lesional (LL) rats. A significant reduction in numbers is shown in the late-lesional rats when compared with pre-lesional and control rats ($0{\cdot}001 < P < 0{\cdot}01$).

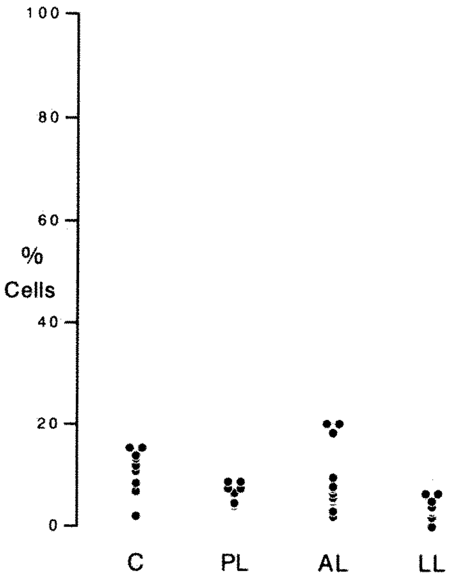

FIGURE 9. Labelling of peripheral monocytes and macrophages from control (C), pre-lesional (PL), actively lesional (AL) and late lesional (LL) rats. Numbers were low in the pre-lesional form ($P < 0{\cdot}02$) and showed an increase during active expression of the lesion. The stable lesion was associated with significantly reduced levels ($P < 0{\cdot}0001$).

and have produced equivocal results. The proportion of T-helper/inducer cells surrounding human follicles is reportedly higher than that of the T-cytotoxic/suppressor cells subset.[3] However, Ranki *et al.*[12] reported a 'concentration' of T-cytotoxic/suppressor cells peribulbarly and perivascularly. Within the lesional whisker follicles of the DEBR rat B lymphocytes and T lymphocytes were observed and a large number of cells were labelled with the monocyte/macrophage marker. Within the skin of alopecia areata patients the presence of B lymphocytes and macrophages has been reported,[12] although numbers of B lymphocytes and monocytes have been reported to be low.[3] These cell types have, however, not been excluded from an important role in the pathomechanism of alopecia areata. Monocytes are reported to show a nearly significant decrease in numbers peribulbarly on treatment with minoxidil.[13]

In the pre-lesional state, the DEBR rat vibrissa follicles expressed class II MHC antigens on the pre-cortical cells. The ectopic expression of MHC antigens is a feature of alopecia areata,[4,5,14] and lends support to the concept of an autoimmune pathomechanism. The aberrant expression of MHC class II antigens has been described for well recognized autoimmune conditions such as Graves' disease.[7] This may be recognized by MHC-restricted T-helper/inducer cells[15] and the consequent release of γ-interferon by these T lymphocytes could cause expression of class II MHC antigens, as observed in the active and later stages of the lesions in the rats. Such an immune mechanism of recognition was first suggested for human alopecia areata by Messenger and Bleehen.[4] The staining observed within the pre-cortical area of the mid-lesional follicles, whilst consistent with the observations made by Messenger and Bleehen,[4] may be an epiphenomenon originally triggered by the aberrant expression of MHC class II antigens on well-differentiated epidermal component of the pre-lesional state. The loss of MHC expression within the pre-cortex of the vibrissa follicles may be due to the dedifferentiation of the epidermal component upon insult. The expression of MHC class II antigens is lost within human alopecia areata follicles when they go into telogen,[14] the state in which they are 'safe' from lesional insult.

In the lesional state the numbers of T lymphocytes were increased when compared with control and pre-lesional rats. This contrasts with the results of peripheral leucocyte counts from human alopecia areata patients where total T-lymphocyte numbers were reported to be normal.[8,10,16] The significantly increased levels of both T-lymphocyte subsets in the lesional form of the DEBR rat over the pre-lesional form indicates the continual proliferation of these cells and their transportation to the skin throughout the course of the lesion. A slight increase in the T-helper/inducer subset was reported by Majewski *et al.*[9] but these workers also found a reduction in the numbers of T-cytotoxic/suppressor cells. However, increases in T-cytotoxic/suppressor levels have also been reported.[3,10]

B-lymphocyte levels showed a reduction during the actively lesional form of the DEBR rat and exhibited a further decrease when the lesion stabilized. One study[17] reported these cells to be at normal levels in human alopecia areata, but another[10] reported reduced levels during lesional expression.

The increase in the concentration of monocytes and macrophages in the peripheral blood of actively lesional DEBR rats may reflect the role of these cells in cytotoxic mechanisms.

It is apparent that differences exist between pre-lesional and control rats which suggest that the DEBR rats may be immunologically predisposed to the expression of the alopecic condition in the pre-lesional form. This may parallel the case for human alopecia areata patients, but there are obvious limitations to the study of the pre-lesional form in man. The DEBR rat may therefore provide an important model system and source of information regarding the pre-lesional state of alopecia areata.

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British Journal of Dermatology (1990) **123**, 569–572.

The presence of autoantibody to recombinant lipocortin-1 in patients with psoriasis and psoriatic arthritis

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SUMMARY

Corticosteroids may mediate some of their anti-inflammatory effects via induction of a specific 38 kDa protein, lipocortin-1. Anti-lipocortin-1 antibodies (ALA) were measured by enzyme-linked immunosorbent assay (ELISA) in 23 patients with plaque-type psoriasis alone (NAP), in 21 patients with psoriasis and arthritis (PA), and in 67 healthy controls. Only two of 23 NAP patients had elevated ALA, whereas six of 21 PA patients had raised levels of ALA ($P = 0{\cdot}2$). Sero-negative polyarthritis was the most common pattern of joint disease in those PA patients with elevated ALA (4/6). ALA levels did not correlate with the extent or severity of cutaneous involvement, and are unlikely to be involved in the pathogenesis of cutaneous psoriasis.

Some of the anti-inflammatory actions of corticosteroids may be mediated by the lipocortin family of proteins by inhibition of phospholipase A_2 (PLA_2). The consequent interference with the release of arachidonic acid from membrane-bound phospholipids could reduce the generation of inflammatory mediators such as leukotrienes, prostaglandins and thromboxanes.[1–11] Patients with rheumatic diseases (systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, and periarteritis nodosum) may have elevated levels of antibodies to lipocortin.[12,13] Although the pathogenetic significance is uncertain, anti-lipocortin-1 antibodies (ALA) may interfere with the action of lipocortin and promote release of arachidonic acid and consequently, the formation of pro-inflammatory prostaglandins, leukotrienes and other eicosanoids.

Prostaglandins and leukotrienes have been implicated in the pathogenesis of psoriasis,[14–17] and psoriatic arthritis is more common in patients with severe skin involvement.[18] Also, PLA_2 levels are raised in psoriatic epidermis.[19–21] Therefore, we examined whether patients with psoriasis (with or without arthritis) may have elevated serum ALA levels, which indirectly may promote the inflammatory process.

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METHODS

Twenty-three consecutive patients with plaque-type non-arthritic psoriasis (NAP) and a similar group of 21 patients with plaque-type psoriasis and clinically and/or radiologically documented psoriatic arthritis (PA) were investigated. A complete cutaneous examination was performed on each subject, and the extent of psoriatic involvement estimated by using the 'rule of nines'.[22]

After informed consent was obtained, 5 ml of venous blood was collected from each subject. The serum samples were then analysed using an enzyme-linked immunosorbent assay (ELISA) to detect serum antibodies directed against recombinant lipocortin-1 (ALA) (Biogen, Cambridge, MA, U.S.A.).[13] A group of 67 healthy controls used to establish the normal range of ALA levels for the assay had a median IgG value of 13 units with an upper range of normal of less than 64 units. The IgM median was 20 units, with an upper range of normal less than 64 units.

The results were analysed by an unpaired t-test or the Fisher's exact test and a finding was considered statistically significant if $P \leq 0\cdot05$.

RESULTS

The NAP group consisted of 23 individuals (15 men and eight women; age range 20–76 years, mean age of 36·3 years) with plaque type psoriasis covering 19·6 ± 2·5% (mean ± SEM) of the skin surface (range 5–45%). The group of PA patients consisted of nine men and 12 women (age range 13–78 years, mean age of 47·9 years) with plaque type psoriasis involving 26 ± 3·5% of the skin surface (range 5–70%).

Everyone in the NAP group demonstrated clearing of their psoriasis with topical treatment (tar, topical steroids, anthralin, or UVB radiation). In contrast, several PA individuals did not respond to conventional topical therapies and required the use of either PUVA, methotrexate, etretinate, razoxane or cyclosporin. No patient received oral corticosteroids. Although the NAP group was younger than the PA group ($P = 0\cdot02$), there was no significant difference between the groups as regards the percentage of skin involved with psoriasis.

Only two NAP subjects had increased levels of IgG-ALA (73 and 141 ELISA units, respectively), and neither had received topical steroids. Both patients lacked mitochondrial, gastric parietal cell, thyroglobulin, thyroid microsomal and smooth muscle antibodies, or rheumatoid and antinuclear factors. Four of the 21 arthritic patients had increased levels of IgG-ALA only (77·7, 85·3, 116·7, and 173 ELISA units, respectively), one had increased levels of IgM-ALA alone (65 ELISA units), while another had increased levels of both IgG-ALA and IgM-ALA (289·7 and 334·2 ELISA units, respectively). The latter individual was the youngest in this study and suffered with symmetrical distal interphalangeal arthritis (antinuclear and rheumatoid-factor negative) even though less than 5% of his skin surface area was involved with psoriasis. None of the PA patients with elevated ALA levels were receiving topical steroids at the time of the study.

Four of the six PA patients with elevated ALA levels had sero-negative polyarthritis. In one of these patients anti-thyroglobulin (1:400 dilution) and anti-thyroid microsomal antibodies (1:102,400 dilution) were detected.

Although IgG-ALA and IgM-ALA were seen more frequently in the PA group, this difference was not statistically significant ($P = 0\cdot2$).

DISCUSSION

Our study has demonstrated that elevated levels of ALA exist in some psoriatic patients with and without arthritis.

As observed by Baker *et al.*,[23] individuals with arthritis were significantly older than those with cutaneous involvement alone, raising the possibility that psoriatic arthritis begins several years after the onset of skin disease.

Analysis of sex, age, extent of cutaneous involvement, and type of arthritis did not identify characteristics associated with elevated ALA levels. However, sero-negative arthritis was present in 4/6 (66%) patients with elevated levels of either IgG- or IgM-ALA. Serum IgM-ALA was detected in 53% (7/13) of rheumatoid arthritis patients,[12] while the dominant class of anti-lipocortin antibodies was found to be IgM-ALA in patients with rheumatoid arthritis and systemic lupus erythematosus.[13] This contrasts with our results where increased IgG-ALA alone was the dominant class of antibody.

The pathogenesis of psoriasis is unknown but the activation of prostaglandins and leukotrienes is thought to be pivotal in the disease process.[14-17] It might be expected that patients with increased ALA levels would develop severe or recalcitrant psoriatic disease. This was not observed; although 80% (5/6) of the PA patients with elevated levels of ALA had required hospital admission for the control of their cutaneous disease, there were other severely affected PA individuals who required hospitalization but did not have elevated levels of ALA. In addition, elevated levels of ALA were seen in arthritic patients with only minimal cutaneous involvement and were not generally seen in the NAP group. These observations suggest that ALA are not specifically related to the unsuppressed generation of prostaglandins and leukotrienes in the cutaneous lesions of psoriatic patients. It remains to be determined whether antibodies to other members of the lipocortin family play a role in the pathogenesis of this disease.[24]

In summary, we have demonstrated the presence of elevated ALA levels in psoriatics with and without arthritis. Sero-negative polyarthritis was the pattern most commonly observed in patients with increased ALA levels, but there was no significant correlation between the level of antibody and the degree of cutaneous involvement or resistance to therapy, indicating that anti-lipocortin antibodies are probably not pathogenic in the development of cutaneous psoriatic plaques.

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Radiobiological studies in the naevoid basal cell carcinoma syndrome

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SUMMARY

The naevoid basal cell carcinoma syndrome is described in a 43-year-old male. The response of dividing fibroblasts from this patient to the lethal effects of ionizing radiation was indistinguishable from that of a normal control: mean inactivation dose ($\bar{D}$) = 1·74 Gy and 1·68 Gy, respectively. However, these cells were defective in the repair of potentially lethal damage ($\bar{D}$ = 2·36 Gy and 3·26 Gy, respectively). The response to radiation of G_0 T lymphocytes derived from the patient was similar to that of the control. The frequency of mutant circulating T lymphocytes resistant to 6-thioguanine was 17×10^{-6} which was elevated when compared with age-matched controls. These results suggest an abnormality in the response of the fibroblasts to ionizing radiation damage.

The naevoid basal cell carcinoma syndrome (BCNS) was first described by Howell and Caro in 1959[1] although it is often called Gorlin's syndrome.[2] It is inherited as an autosomal dominant condition with a penetrance of approximately 97%.[3] The characteristic features are the early onset of basal-cell carcinomata,[3] bony anomalies, a typical facies with frontal bossing of the skull and a broad nasal root, palmoplantar pits and cutaneous cysts.[4] However, there are many manifestations of the condition.

Presentation may occur in childhood as a result of skeletal anomalies such as spina bifida, or more rarely due to the development of tumours such as medulloblastomata[5] or ovarian fibromas.[6] At this age basal cell carcinomata are usually seen, but these may be difficult to distinguish clinically from melanocytic naevi.[4] In 50% of patients, the presenting problem is that of jaw cysts,[4] which are usually keratocysts.

We describe a patient with BCNS, and report the results of cellular repair studies on cultures of fibroblasts and lymphocytes derived from this patient.

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PATIENT AND METHODS

Case report

A 43-year-old male had the classical clinical features of the basal cell naevus syndrome. He had radiological evidence of an abnormal rib cage, and had had several jaw cysts removed. At presentation in 1988, he had multiple pigmented basal cell carcinomata of the face and upper trunk. Most of these were cystic basal cell carcinomata, although there was a morphoeic lesion in the left nasolabial fold (Fig. 1). Numerous small pigmented lesions on the upper back were biopsied and these were basal cell carcinomata. He had typical palmoplantar pits. He also had facial dysmorphism, characteristic of BCNS. The patient underwent micrographic surgery to a lesion in his left nasolabial fold and the remaining smaller basal cell carcinomas were removed by local excision, or by electrodessication and cautery.

Cellular repair studies

Fibroblasts. A fibroblast cell strain designated BCNS1BR was established from an explant culture using standard procedures.[7] Two classes of cell survival experiments were performed following ^{60}Co γ-irradiation. In the first, the response of asynchronously dividing cells of BCNS1BR was compared with those of 1BR.3, a standard normal control culture.[8] The second class of experiments compared the ability of BCNS1BR cells with that of 1BR.3 to repair

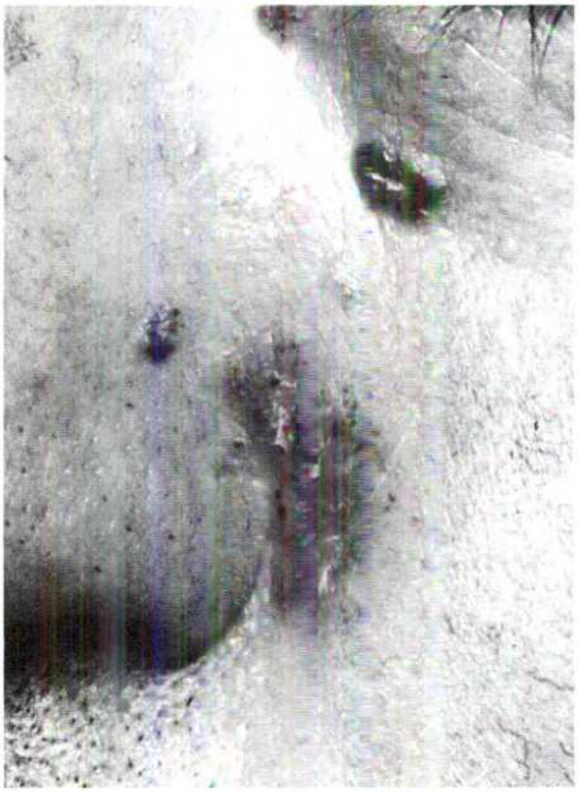

FIGURE 1. Large basal cell carcinoma in the left nasolabial fold.

potentially lethal damage (RPLD) following γ-irradiation. This required an assessment of the radioresistance of the cells taken out of the division cycle by maintenance in a serum-depleted medium both with and without a period of 24 h for repair.[9] All results were evaluated using a National Algorithm Group subroutine by computer and the best fit survival curve determined.[10,11] The mean inactivation dose, $\bar{D}$, was computed for each survival curve for both fibroblasts and lymphocytes to facilitate comparison of the response of the two cell types.

Lymphocytes. The mononuclear cell fraction was separated from 50 ml whole blood using standard procedures[12,13] and designated BCNS1BR TL. Freshly isolated cells were used to assess the radiation dose response of T lymphocytes as previously described,[12] and also the frequency of mutants with resistance to 6-thioguanine.[13] The remaining mononuclear cells were stored in liquid nitrogen[13] to be thawed at a later date, when both experiments were repeated. We have previously demonstrated that the use of fresh or frozen cells does not show a significant difference in the results.[13] The results were again evaluated by computer using a National Algorithm Group subroutine.

RESULTS

Fibroblasts

The survival curves for asynchronously dividing fibroblast cells, BCNS1BR (patient) and 1BR.3 (control), are shown in Figure 2. In agreement with previous results,[8,14] the survival of

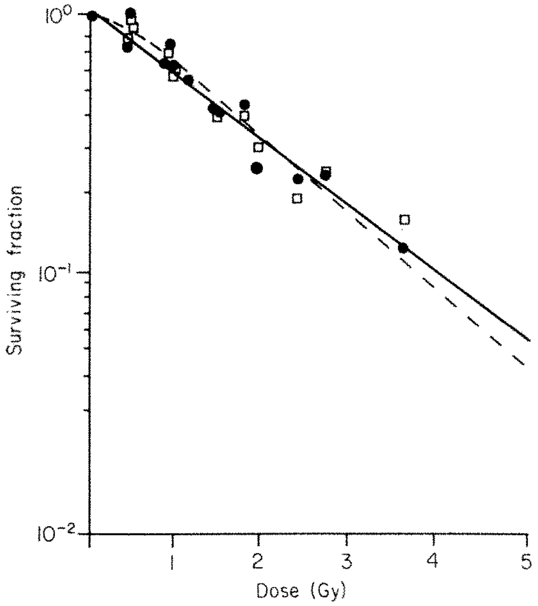

FIGURE 2. The γ-ray survival curve for normal (1BR.3) fibroblasts (□) and fibroblasts derived from the patient (BCNS1BR, ●). These curves were determined in triplicate in paired experiments. $\bar{D}$, determined as described in the text, was 1·68 Gy for 1BR.3 and 1·74 Gy for BCNS1BR. The plating efficiencies for 1BR.3 were 59, 100 and 19% and for BCNS1BR they were 93, 23 and 61% in the same paired experiments.

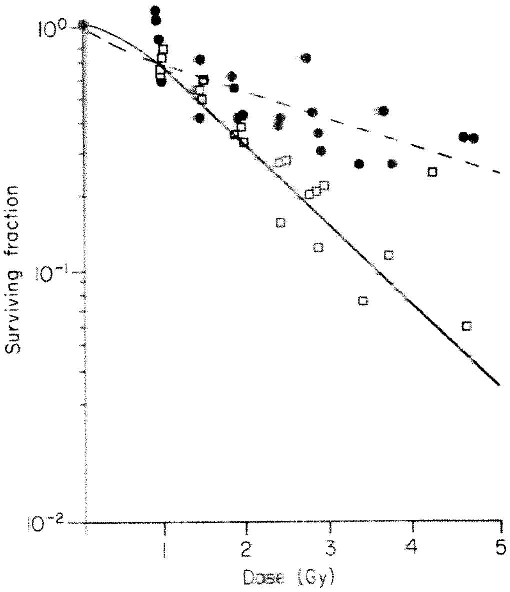

FIGURE 3. Repair of potentially lethal damage in a normal cell strain (1BR.3). 1BR.3 C (□) = survival of control culture, on immediate release from serum depletion, $\bar{D}$ = 1·78. 1BR.3 LH (●) = survival after a 24-h period for the repair of potentially lethal damage, $\bar{D}$ = 3·26 Gy. The plating efficiencies for the control cultures were 59, 24, 51 and 38% and after the repair period were 69, 22, 57 and 27% in the same order.

the BCNS1BR cells was indistinguishable from that of normal cells. The cloning efficiencies and survival curve parameters are given in the legend to Figure 2.

The results of a series of four experiments, in which experiments with BCNS1BR cells were paired with experiments with 1BR.3 cells, show that the cells derived from our patient had a limited capacity to repair RPLD (Figs 3 and 4). The level of repair was not as substantial as in 1BR.3 as the survival curve parameter $\bar{D}$ was increased from 1·73 Gy to 2·36 Gy in BCNS1BR compared with an increase from 1·78 Gy to 3·26 Gy in 1BR.3.

The ratio of the two $\bar{D}$ values for BCNS1BR was 1·4 and 1·8 for 1BR.3. In a separate study the relevant ratios for three normal fibroblast cultures were: 1·6, GM730 (seven experiments); 1·7, 70BR (four experiments) and 1·8, 1BR.2 (a second independent biopsy of donor 1BR) (19 experiments).

Lymphocytes

The radiation survival curves for the G_0 T lymphocytes derived from our patient (BCNS1BR TL) compared to G_0 lymphocytes from three normal adult donors are shown in Figure 5. The following $\bar{D}$ values were obtained: 2·16 Gy for BCNS1BR TL, and 1·83, 1·67 and 1·92 Gy for normal donors 1, 2 and 3, respectively. The result for BCNS1BR TL lay within the normal range for T lymphocytes as determined in our laboratory.[12]

The frequency of mutants with resistance to 6-thioguanine among the BCNS1BR TL cells was elevated, being $17{\cdot}71 \times 10^{-6}$ (mean of two experiments), when compared to our normal donor range.[13] Since analysis using a single donor is not appropriate, the results from BCNS1BR TL and two other BCNS donors (mutant frequencies $13{\cdot}97 \times 10^{-6}$ and $6{\cdot}4 \times 10^{-6}$)

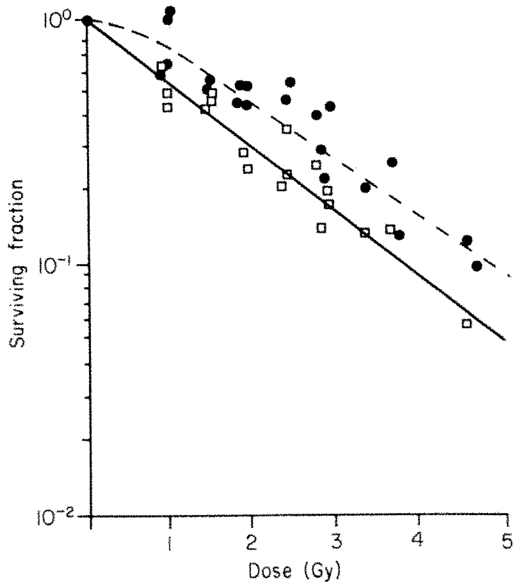

FIGURE 4. Repair of potentially lethal damage in BCNS1BR, BCNS1BR C (□) = control culture, on immediate release from serum block, $\bar{D}$ = 1·73 Gy. BCNS1BR LH (●) = BCNS1BR survival after a 24-h period for the repair of potentially lethal damage, $\bar{D}$ = 2·36 Gy. The plating efficiencies for the control cultures were 19, 87, 64 and 41% and after the repair period were 15, 86, 79 and 49% in the same order and paired with the results for 1BR.3 in Figure 3.

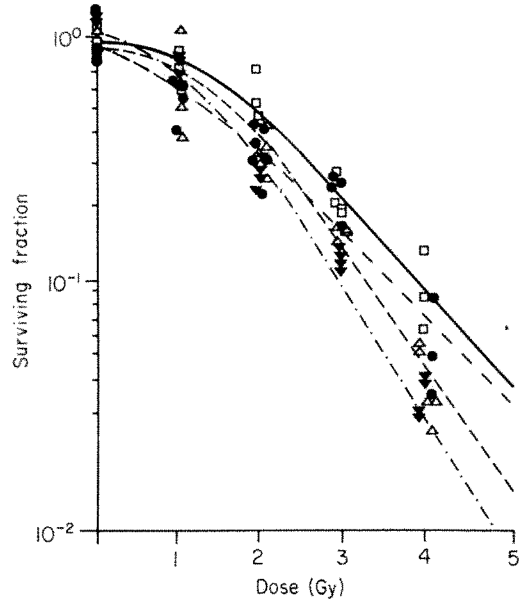

FIGURE 5. The γ-ray survival curves for T lymphocytes from three normal donors (1●, $\bar{D}$ = 1·83 Gy; 2▼, $\bar{D}$ = 1·67 Gy, 3△, $\bar{D}$ = 1·92 Gy) and BCNS1BR TL (□, $\bar{D}$ = 2·16 Gy). 2 experiments per donor. Mean cloning efficiency for BCNS1BRTL was 55·5%, and for the normal donors 18, 29 and 72%, respectively.

were compared to our normal donor data base using the computer program GLIM.[12] Taking age and smoking habits into account, the BCNS group was found to have an elevated, but not significantly high, mutant frequency.

DISCUSSION

In general, the vast majority of the cutaneous basal cell lesions remain benign,[15] and radical treatment is inappropriate.[4] However, destructive malignant transformation may occur, especially on the head and neck, and therefore more radical surgical treatment is recommended for basal cell carcinomata (BCC) at high risk sites. In our patient a large BCC in the nasolabial fold required treatment with micrographic surgery.

In the basal cell carcinoma syndrome (BCNS) there is an inherited predisposition to malignancy. It has been suggested that the carcinogenesis is a multistage process,[3] and that 'at risk' patients will develop a tumour when a second environmental event occurs. For instance, ionizing radiation undoubtedly may act as a second trigger and therefore is absolutely contraindicated in the affected patients. Children given irradiation to the skull for medulloblastoma have developed multiple BCC in the irradiation field only 6–36 months after radiotherapy in contrast to the usual latency of 21 years.[5] Similarly affected patients given irradiation to the hands for eczema have developed multiple BCC on the palms and dorsa of the hands.[16]

While there is no proof that ultraviolet light (UV) is the second factor in carcinogenesis, it does seem probable based on cellular studies[17] and the distribution of the tumours on the head and neck. Therefore, advice to the patients to limit UV exposure and to use sun-screens would be appropriate.

There have been several attempts to define the mechanisms underlying this syndrome. Chromosomal studies using modern banding techniques have failed to demonstrate an anomaly.[18] Lymphocytes from affected patients have a significantly higher level of X-irradiation-induced chromosome aberrations.[19] The results of DNA repair studies on fibroblasts and lymphocytes have been confusing. The γ-irradiation sensitivity of asynchronously dividing fibroblasts from three BCNS patients was shown to be indistinguishable from that of normal individuals.[8,14] Slight hypersensitivity to X-ray-induced lethality was reported in fibroblasts[20] and confirmed in a further series of studies where hypersensitivity to the lethal effects of X-rays, UV-light and mitomycin C was found.[21] We have previously examined the sensitivity of three BCNS fibroblast cell strains to UVC and found no difference from normal controls.[22] However, Ananthaswamy *et al.* have claimed a sixfold enhancement of sensitivity for BCNS fibroblasts compared with normals to UVB but not UVC.[17] The ionizing radiation response of both asynchronously dividing fibroblasts and G_0 T lymphocytes from the patient under investigation was normal. Skin-derived keratinocytes from both BCNS and normal donors were more resistant to UVC and X-rays than skin-derived fibroblasts.[23] No difference in survival between BCNS and normal cells to either DNA damaging agent was observed.

These ionizing radiation studies were extended to include an investigation of RPLD[19] and again no difference could be distinguished between fibroblasts from BCNS patients and controls. However, Arlett and Priestley[24] tested three fibroblast stains and showed that in two, BCNS1B1 and BCNS3B1, there was very little capacity for RPLD while the third, BCNS2B1 I showed some repair capacity. These results indicated heterogeneity in BCNS with respect to RPLD, and these patients may be defective in RPLD of ionizing radiation damage although clearly this is not diagnostic.[24]

The frequency of 6-thioguanine-resistant mutants in circulating T lymphocytes has been measured in a peripheral blood sample from the patient under investigation, and was shown to be elevated for the age. While an elevated mutant frequency is not directly correlated with the propensity to or the incidence of carcinoma, it is seen in patients suffering from the cancer-prone syndromes, xeroderma pigmentosum and ataxia telangiectasia.[25,26] This may be of significance in BCNS. Clearly more studies are required in a larger series of patients with BCNS.

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Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin

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SUMMARY

Ornithine decarboxylase (ODC) was measured in the epidermis of healthy volunteers and the uninvolved skin of psoriatic patients at various times after sellotape stripping. Basal levels were less than 1 pmol/min/mg protein. Activity peaked to a maximum of 86 pmol/min/mg protein after 8 h; this was followed by an abrupt decline to a lower level which remained relatively constant for at least 36 h. No difference was seen between the response of controls and psoriatic patients. Pretreatment with topical corticosteroids reduced peak ODC levels to about one-half, but oral indomethacin had no effect.

Ornithine decarboxylase (ODC) is the rate-limiting enzyme for polyamine biosynthesis. Cellular ODC levels are linked to the phase of the cell cycle. Quiescent or early G_1 cells show low activity, but there is an abrupt increase in late G_1 which provides the polyamines that are required for DNA synthesis. Thus, ODC is a valuable 'marker enzyme' for cellular proliferation.[1,2]

Most of the published investigations regarding epidermis have used rodents, where ODC induction has been demonstrated following injury (e.g. tape-stripping[3] or ultraviolet radiation[4–9]) or the application of tumour-promoting agents such as tetradecanoylphorbol acetate (TPA).[10–12] Several groups have reported increased ODC levels in the lesions of psoriasis, and shown that these normalize following therapy.[2] More recently, it has been claimed that ODC is 'hyper-inducible' in the psoriatic patient.[13] However, to our knowledge the time-course of ODC induction following injury has never been investigated in human skin. Here we report ODC levels following tape-stripping in healthy controls and psoriatic patients, and describe the effect of pretreatment with topical corticosteroids or oral indomethacin.

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METHODS

Subjects

The control group consisted of 19 volunteers (10 male, nine female; age range 21–57 years), none of whom had a personal or family history of psoriasis. The psoriatic group comprised eight patients (five male, three female; age range 17–55 years) with chronic plaque psoriasis, who had received no therapy for at least 4 weeks prior to investigation. The use of aspirin or other NSAIDs was specifically excluded in all subjects during or immediately prior to experiments. Informed consent was obtained from all the subjects and the study had the approval of the Medical Ethics Committee.

Sellotape stripping was carried out as described previously,[14] using a plastic template to restrict the damage to sharply-defined areas of 2 × 3 cm, and the stripped sites covered with a non-occlusive dressing. At appropriate times, epidermal slices of approximately 1 cm^2 were cut from the centre of each site using a Castroviejo keratotome (Storz Instrument Co.) set for a depth of 0·3 mm after cooling the skin with an ethylchloride spray. Biopsies were snap frozen in liquid nitrogen and kept at this temperature prior to analysis.

Six volunteers were pretreated with oral indomethacin, taking 75 mg 14 h and 2 h prior to tape-stripping. A second group, consisting of five volunteers were pretreated with either topical betamethasone dipropionate or a base cream under occlusion for 14 h prior to stripping. The sites were thoroughly cleaned with 70% alcohol immediately prior to stripping, and biopsies taken for ODC measurement after a further 8 h.

ODC measurement

This was a modification of a previously described method.[15] Biopsies were homogenized in 0·5 ml of a freshly prepared buffer (50 mM Tris, 50 μM pyridoxal phosphate and 2 mM dithiothreitol at pH 7·3) using an ice-cooled Potter-type grinder. The reaction was initiated by adding 80 μl of homogenate to 20 μl of a solution containing 2 mM L-ornithine and 0·1 μCi L-[1-^{14}C]ornithine (New England Nuclear, Boston, MA, U.S.A.) in plastic tubes. After fitting each tube with a plastic cap carrying 5 cm^2 filter paper moistened with 20 μl 10% KOH, the mixture was incubated at 37°C for 45 min. The reaction was stopped by adding 100 μl 1M HCl, and incubated at 37°C for a further 30 min to ensure complete absorption of $^{14}CO_2$ on to the paper. Radioactivity was measured by scintillation counting using an Isocap-300 (Searle Instruments B.V.); the paper was placed directly into a scintillation vial containing 4·5 ml scintillation fluid (Aqua–Luma Plus) without prior dissolution. All samples were assayed in duplicate, and a complete reagent blank was included with each batch.

Preliminary experiments were carried out as described above to verify the linearity of the reaction with respect to time for incubation periods up to 60 min.

Protein measurement

An aliquot of the homogenate was diluted 1:5 with homogenizing buffer and centrifuged (10 min, 15,000 ***g***). Protein was determined in the clear supernatant by direct fluorescence ($\lambda_{EX} = 278$ nm, $\lambda_{EM} = 340$ nm) using bovine serum albumin as standard. Linearity was confirmed in the range 1–200 μg/ml protein.

Statistical methods

Analysis of variance was carried out for time and diagnosis using the SAS package and a VAX computer system.

RESULTS

The total reagent blank (including instrument background) averaged about 20 c.p.m. If an increment of 5 c.p.m. above this level is regarded as the minimum required to detect ODC activity, and assuming a typical value of around 250 μg/ml for the concentration of soluble protein in the original homogenate, the absolute sensitivity of the method may be calculated to be about 1 pmol CO_2 released per min per mg protein. The assay was linear with respect to time for about 45 min, but showed a clear decline at 1 h. An incubation period of 45 min was therefore used for routine measurements. The reproducibility was good and duplicate assays averaged within ± 4·3% of the mean.

No ODC activity could be detected in biopsies taken immediately after stripping (i.e. less than 1 pmol/min/mg protein). The time-curve during the following 36 h is shown in Figure 1. An abrupt increase at 4 h reached a peak of 86 pmol/min/mg protein at 8 h. This was followed by an equally rapid diminution, reaching a level which remained relatively constant until at least 36 h post-stripping. The 24-h specimens, although averaging rather higher than the 16 h and 36 h values, did not differ significantly from the latter groups. No significant difference in ODC activity was observed between healthy volunteers and the clinically uninvolved skin of psoriatic patients at any of the times studied (4, 8 and 16 h).

The peak ODC levels in sites pretreated with betamethasone dipropionate averaged 45 pmol/min/mg protein, around one-half the value of either the untreated controls or the sites which had been occluded with base only (Table 1). By contrast, subjects receiving oral indomethacin prior to biopsy did not show any significant difference from the control group.

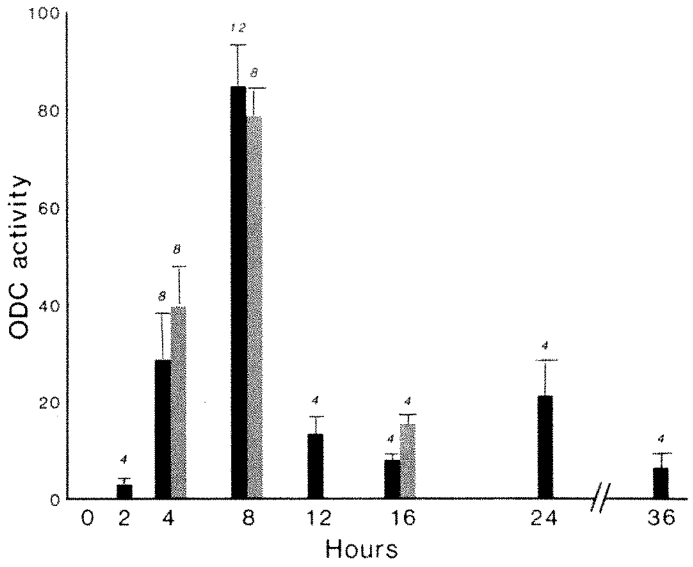

FIGURE 1. ODC levels in healthy (■) and psoriatic uninvolved (▨) epidermis at various times after sellotape stripping. All values are expressed as pmol/min/mg protein (mean ± SE). Numbers of specimens are shown at the top of the figure.

TABLE 1. Effects of pretreatment with indomethacin or betamethasone dipropionate on epidermal ODC measured 8 h after tape stripping. Values are expressed as pmol/min/mg protein (mean ± SE). The control values for the indomethacin experiment are from Figure 1; for the betamethasone dipropionate experiment the control sites were pretreated with base cream (betamethasone dipropionate vs. control, $P < 0.05$)

Treatment	Experimental group	Control group
Indomethacin	82 ± 8 ($n = 6$)	86 ± 8 ($n = 12$)
Betamethasone dipropionate	45 ± 7 ($n = 5$)	87 ± 5 ($n = 5$)

DISCUSSION

The time-course of ODC induction in human subjects following stripping was similar to that following the application of tumour-promoting agents to mice, where enzyme activities peak around 6–10 h.[10–12] A similar value of 6 h has been reported following stripping the skin of the hairless rat,[3] but peak ODC levels were seen 12–28 h following treatment of mice with UVB.[4,5,8,9] Absolute values in rodent epidermis show wide variation, probably due to differences in methodology but most groups report basal ODC activity as being too low to measure.

Only one previous report has appeared regarding the effect of injury on ODC levels in human subjects.[13] These workers used an arbitrary induction period of 6 h, and compared the response of psoriatic patients to sellotape stripping with that of healthy controls. Their findings differ in several respects both from previous work and to our present observations. All specimens showed remarkably high basal levels, no significant difference being reported between psoriatic lesion and normal skin. At 6 h, ODC activity had doubled in healthy subjects, but in psoriatic patients had risen by a factor of five- to 10-fold. By contrast, our data (Fig. 1) indicate at least an 80-fold stimulation which is identical in normal and psoriatic uninvolved skin. The reasons for these discrepancies are not clear. The only significant methodological difference is that the previous workers do not mention correcting their data for a reagent blank; this could account for the higher basal activity and hence the lower induction ratios.

There is considerable evidence that an arachidonic acid metabolite (presumably a cyclo-oxygenase product) is required for ODC induction. Thus, the response to stimuli such as TPA applied to the skin of rodents is blocked by corticosteroids or cyclo-oxygenase inhibitors.[16–19] However, this data is difficult to extrapolate to human skin, especially using appropriate routes of administration and dosage schedules. We have shown in this study that betamethosone dipropionate results in a clear reduction of ODC levels following injury (Table 1). Thus, in addition to their anti-inflammatory and other effects, corticosteroids may diminish proliferation via a reduction in polyamine availability.

The induction time following stripping was similar to that reported for TPA. Since the induction of ODC is mediated via the activation of protein kinase C,[12] either directly (by TPA) or indirectly following injury, we conclude that the sequence of events between injury and protein kinase C activation is fairly rapid. Thus, the long delay between stripping and G_0 recruitment[20] occurs 'distal' to protein kinase activation, and cannot be explained by the slow release or diffusion of cytokines resulting from stripping. Also, because the first wave of DNA synthesis begins around 35 h after stripping,[20] what is the function of polyamines peaking virtually a full cell cycle before this time? The reasons for this await further research.

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The significance of Leu 8 negative T cells in lymphoid skin infiltrates: malignant transformation, selective homing or T-cell activation?

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SUMMARY

The expression of Leu 8 was studied on skin biopsies from a large group of patients with benign and malignant skin disorders and correlated with the expression of T-cell differentiation antigens and activation markers. The effect of *in vitro* stimulation of peripheral blood T cells and T-cell subsets on the expression of Leu 8 antigen was also determined. In all the skin diseases studied an inverse relationship was found between the proportions of cells expressing Leu 8 and HLA-DR. A deficiency of Leu 8 positive cells was not specific for mycosis fungoides, but was also found in several reactive dermatoses. Stimulation of peripheral blood cells with phytohaemagglutinin (PHA), concanavalin A (Con A), and anti-CD3-PMA resulted in a considerable decrease of Leu 8 antigen expression on day 3 in both $CD4^+$ and $CD8^+$ T cells. These data suggest that the low proportion of Leu 8^+ T cells in mycosis fungoides and several reactive skin disorders is not related to malignant transformation or selective homing of Leu 8^- T cells, but probably results from local T-cell activation.

The monoclonal antibody Leu 8 reacts with a cell-surface antigen present on approximately 80% of $CD4^+$ T cells and 60% of $CD8^+$ T cells.[1] Thus, approximately 70% of the circulating T cells express the Leu 8 antigen. In skin biopsies from several dermatoses, including discoid lupus erythematosus (DLE) and mycosis fungoides (MF) a much lower proportion of Leu 8 expressing T cells has been found.[2–5] The cause of this is as yet unknown. Selective infiltration of Leu 8^- T cells into the skin[3] and loss of Leu 8 antigen during malignant transformation[4,5]

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have been suggested as possible explanations for this Leu 8 deficiency. In a recent immunohistochemical study of skin biopsies of patients with Jessner's lymphocytic infiltrate of the skin (LIS), polymorphous light eruption (PLE) and DLE we found considerable differences in the number of Leu 8^+ cells.[6] In addition, our results suggested a relationship between Leu 8 deficiency and T-cell activation. The aim of the present study was to find out whether Leu 8 deficiency was correlated with or perhaps the result of T-cell activation. Therefore, Leu 8 expression was studied on skin biopsy specimens from a large group of patients with both benign and malignant skin disorders and correlated with the expression of T-cell differentiation antigens and activation markers. In addition, the effect of *in vitro* stimulation of peripheral blood T cells and T-cell subsets on the expression of Leu 8 antigen was determined.

METHODS

Patients

Skin biopsies were obtained from six patients with plaque-stage mycosis fungoides (MF), five patients with chronic dermatitis, six patients with lichen planus, 10 patients with discoid lupus erythematosus, six patients with Jessner's lymphocytic infiltrate of the skin, 12 patients with psoriasis and six patients with polymorphous light eruption (PLE). The patients received no treatment during the 2 weeks prior to biopsy. Only those patients with characteristic clinical and histological findings of their disorder were included. Immunofluorescence studies showed a positive lupus band test in all the cases of DLE and immunofluorescence and serological studies were negative in all patients with LIS and PLE.

Immunohistochemistry

Punch biopsies from lesional skin of all patients were snap frozen in liquid nitrogen and stored at −90°C until used. Acetone-fixed cryostat sections were processed using an indirect immunoperoxidase technique. The monoclonal antibodies anti-CD2, CD3, CD4, CD5, CD8, CD11c, CD22, CD25, CD71, Leu 8 and HLA-DR were used as the primary antibody (Table 1) and for the second step a horseradish peroxidase-labelled rabbit anti-mouse immunogobulin conjugate

TABLE 1. Monoclonal antibody panel

CD	Antibody	Source*	Specificity	Dilution
CD2	Leu 5	BD	Pan T cell	1:50
CD3	Leu 4	BD	Pan T cell	1:50
CD4	Leu 3	BD	Helper/inducer T-cell subset	1:10
CD5	Leu 1	BD	Thymocytes, mature T cells	1:100
CD8	Leu 2	BD	Cytotoxic/suppressor T-cell subset	1:50
CD11c	Leu M5	BD	Macrophages	1:50
CD22	Leu 14	BD	Pan B cells	1:50
CD25	anti-IL2R	DAKO	Activated T/B cells	1:10
CD71	OKT9	Ortho	Transferrin receptor	1:25
—	Leu 8	BD	'Immunoregulatory' T and B cells	1:25
—	HLA-DR	BD	B cells, activated T cells, macrophages	1:25

* BD, Becton & Dickinson, Sunny Vale, CA, U.S.A. Ortho, Ortho Diagnostics, Raritan, NJ, U.S.A. DAKO, Dakopatts, Copenhagen, Denmark.

was used.[7] The incubation time for both the first and second step was 30 min. Peroxidase was revealed using diaminobenzidine and hydrogen peroxide. The sections were counterstained with haematoxylin and embedded in malinol. The percentage of positively stained cells was estimated semiquantitatively by two independent observers.

Cell activation studies

Peripheral blood mononuclear cells (PBMC) obtained from healthy donors were isolated on Ficoll–Hypaque. T cells were isolated after rosetting with neuramidase-treated sheep red blood cells. CD4$^+$ and CD8$^+$ cells were isolated by immunomagnetic separation of T cells. To isolate CD4$^+$ cells, T cells were incubated for 30 min with anti-CD8 and anti-Leu 19 monoclonal antibodies. The T-cell suspension was depleted of CD8$^+$ and Leu 19$^+$ cells using sheep anti-mouse immunoglobulin labelled magnetic beads according to the manufacturer's instructions (Dynabeads, Oslo, Norway). The resulting unlabelled cells were used for activation studies and on FACS analysis were >95% CD4$^+$. The CD8$^+$ cell suspensions were isolated using a comparable method. These suspensions contained >90% CD8$^+$ cells.

Cells were cultured in RPMI supplemented with 10% fetal calf serum for 3 days. For activation studies, PHA (2·5 μg/ml), Con A (5 μg/ml) or monoclonal anti-CD3 (ascitic fluid, 1 : 10·6) with phorbol myristate acetate (1 ng/ml) were added to the culture media. Unfixed cells were analysed for marker expression by FACS, after incubation at 0°C with appropriate dilutions of FITC or phycoerythrine-labelled antibodies. Statistical analysis was performed using the *t*-test for paired comparisons.

RESULTS

Immunohistochemistry

In all the biopsies there was a predominance of CD3$^+$ T cells, whereas CD22$^+$ B cells were few or absent. The results of staining with monoclonal antibodies against Leu 8 and HLA-DR antigens are shown in Figure 1. The lowest proportions of Leu 8$^+$ cells were found in MF (mean

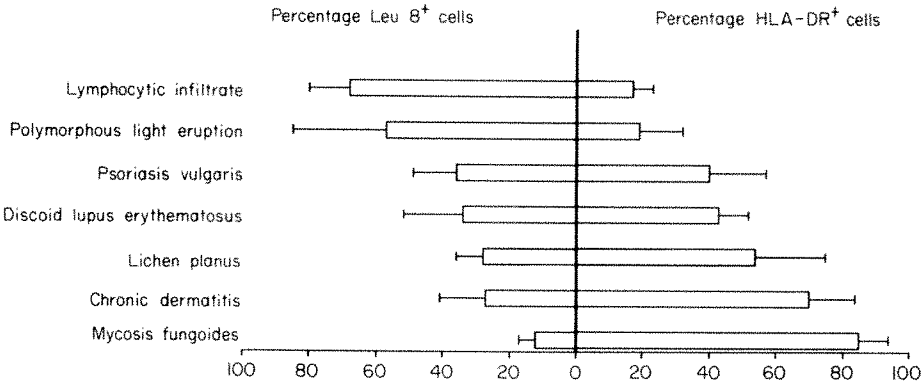

FIGURE 1. The proportions of Leu 8$^+$ and HLA-DR$^+$ cells in the dermal infiltrates of patients with mycosis fungoides ($n = 6$), chronic dermatitis ($n = 5$), lichen planus ($n = 6$), discoid lupus erythematosus ($n = 10$), psoriasis vulgaris ($n = 12$), polymorphous light eruption ($n = 6$) and Jessner's lymphocytic infiltrate ($n = 13$). Shown are the mean ± SE.

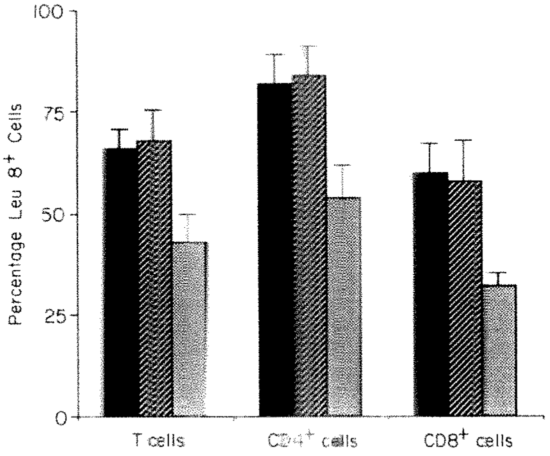

FIGURE 2. Expression of Leu 8 on cultured circulating T-cell subsets: day 0 (■), day 3 without PHA stimulation (▨) and day 3 with PHA stimulation (▩), measured by flow cytometry. The mean percentage of Leu 8^+ cells ± SE is shown ($n = 4$).

12%, range 5–20%). In all six cases more than 75% of the infiltrating cells expressed HLA-DR antigens. The highest proportions of Leu 8^+ cells were found in LIS (mean 68%, range 40–80%) and PLE (mean 57%, range 20–80%). In only three biopsies was Leu 8 expression found on less than 60% of the cells. In both LIS and PLE the staining with anti-HLA-DR was largely confined to macrophages and endothelial cells, whereas the lymphocytes were generally not reactive. In the other dermatoses studied, the percentages of Leu 8^+ cells varied between 10 and 60% and in 21 of 33 biopsies the percentage of Leu 8^+ cells was lower than 30%. HLA-DR expression varied from 20 to 90%. In only nine of 33 biopsies was the proportion of HLA-DR^+ cells 60% or more.

Our results have demonstrated an inverse relationship between the proportions of Leu 8 and HLA-DR expressing cells (Fig. 1). This is further substantiated by the finding in one case of DLE and one case of lichen planus of perivascular infiltrates with high proportions of Leu 8^+, HLA-DR^- T cells, next to other infiltrates with high proportions of Leu 8^-, HLA-DR^+ T cells in the same section. There was no relationship between the relative proportions of $CD4^+$ and $CD8^+$ T cells and the number of Leu 8^+ cells. In one case of MF there was a predominance of $CD8^+$ T cells (c. 70%) while only 20% of the T cells expressed Leu 8. In all the biopsies studied, CD25- and CD71-expressing cells were few (5–10%) or absent and no differences were found between the diseases studied.

Cell activation studies

There was a significant decrease in Leu 8 antigen expression on day 3 in PHA-stimulated T cells as measured by flow cytometry ($P < 0.001$). This decrease was observed in both $CD4^+$ ($P < 0.005$) and $CD8^+$ ($P < 0.001$) T cells (Fig. 2). Similar reductions in the proportions of Leu 8^+ T cell subsets were seen after stimulation with Con A and anti-CD3 with PMA (Fig. 3). In PHA-stimulated T cells the decline of Leu 8 expression was paralleled by a marked rise of CD25-expressing T cells on day 3.

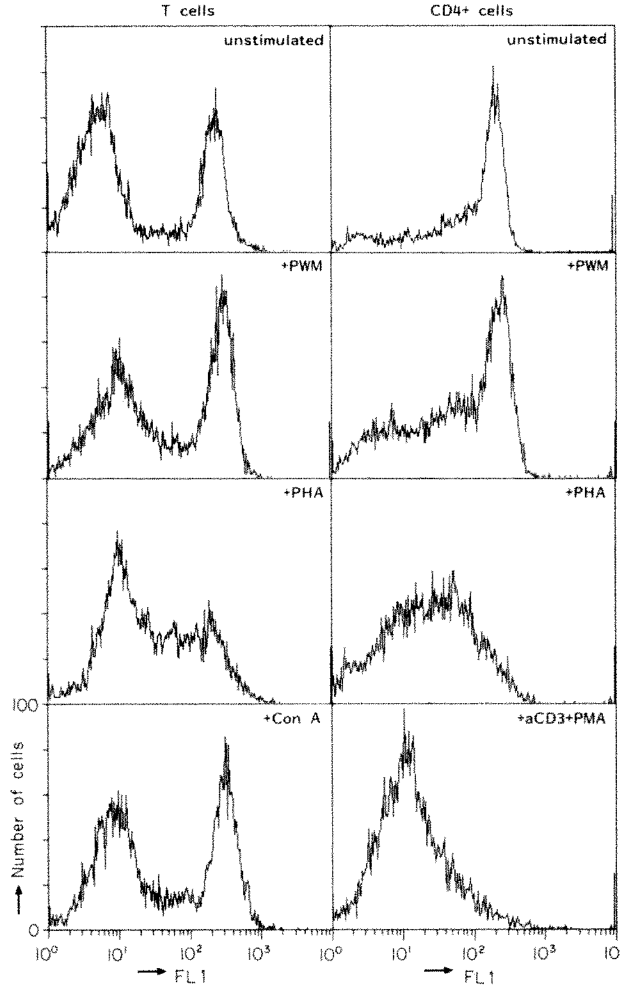

FIGURE 3. FACS analysis of Leu 8 expression on peripheral blood T cells and CD4$^+$ cells after 3 days' culture.

DISCUSSION

At present there is no generally accepted explanation for the low numbers of Leu 8 expressing T cells as observed in skin infiltrates of various benign and malignant dermatoses. This Leu 8 deficiency was first described in patients with mycosis fungoides (MF) by Abel *et al.*[4] who proposed two possible explanations. The neoplastic cells in MF might derive from a Leu 8$^-$ T-cell subset at high risk for malignant transformation, or the Leu 8 antigen might be lost during malignant transformation.[4] In extended studies the same group noted that deficiency of Leu 8 and Leu 9 antigens was much more prevalent in MF than in various benign dermatoses.[5] It was therefore suggested that determination of the percentages of Leu 8$^+$ and Leu 9$^+$ cells may be a useful criterion to differentiate between MF and cutaneous inflammation.[5] However, subsequent studies demonstrated low numbers of Leu 8$^+$ T cells in several benign conditions as well, indicating that Leu 8 expression is not specifically linked to malignant transformation and can not be considered as a reliable diagnostic criterion.[2,3,6,8] In the present study low numbers of Leu 8$^+$ T cells were found not only in MF, but also in several reactive skin disorders. In chronic dermatitis, lichen planus, discoid lupus erythematosus and psoriasis vulgaris we found 30% or less Leu 8 expressing cells in 21 of 33 (64%) of the skin biopsies. In 1987 Ashworth *et al.* reported low proportions of Leu 8$^+$ cells in the dermal infiltrates of patients with DLE.[3] They suggested that the Leu 8 deficiency in the skin of DLE might reflect selective infiltration of Leu 8$^-$ T cells in this condition.

The results of the present study suggest that there may be another explanation for the Leu 8 deficiency as observed in the skin infiltrates of various benign and malignant dermatoses. We found a clear-cut inverse relationship between the proportions of Leu 8$^+$ T cells and HLA-DR$^+$ T cells, generally considered to represent activated T cells. In biopsies from patients with LIS and PLE, with high numbers of Leu 8$^+$ T cells, the endothelial cells and macrophages strongly expressed HLA-DR antigens, but HLA-DR$^+$ T cells were few or absent. In contrast, in MF there were very low numbers of Leu 8$^+$ T cells and the majority of T cells expressed HLA-DR antigens. In the other conditions studied intermediate numbers of Leu 8$^+$ and HLA-DR$^+$ T cells were present (see Fig. 1). In addition, in skin biopsies from one patient with DLE and one with lichen planus the perivascular infiltrates showed a high proportion of Leu 8$^+$ and HLA-DR$^-$ T cells, next to infiltrates in other parts of the same biopsy with a predominance of Leu 8$^-$, HLA-DR$^+$ T cells.

A similar inverse relationship between HLA-DR and Leu 8 expression can be found in reactive lymph nodes and in the tonsils. In the paracortical T cells about 80% of the T cells are Leu 8$^+$, while these cells do not generally express HLA-DR antigens.[9] These findings suggest that the differential staining of T lymphocytes for Leu 8 in various skin disorders may reflect the state of activation of these T cells in the skin infiltrates. Our *in vitro* studies that showed a decrease of Leu 8 expression on T cells after stimulation with mitogens provide further evidence for the relationship between Leu 8 expression and T-cell activation. Our results are in keeping with others, who have found a decline of Leu 8 expression from 57 to 12% after stimulation of peripheral blood lymphocytes with PHA.[10] This occurred, as in our study, with the expected increase in CD25 expression.

It is tempting to speculate that Leu 8 deficiency in cutaneous lymphoid infiltrates results from local T-cell activation of the infiltrating T cells. However, selective infiltration of activated Leu 8$^-$ T cells into the skin cannot be excluded. Several studies have demonstrated that activated T cells adhere much better to both endothelial cells and keratinocytes than unstimulated T cells.[11,12] Furthermore, the recent finding that the Leu 8 antigen is homologous to the homing receptor Mel-14 is of interest,[13] as Mel-14 mediates the homing of periheral blood lymphocytes in peripheral lymph nodes. Further studies to define the role of Leu 8/Mel-14 in the homing of T lymphocytes in the skin are in progress.

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Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol

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SUMMARY

In this study, the nature of non-immune immediate contact reactions (NIICR) produced by cinnamic aldehyde, benzoic acid and sorbic acid were investigated, with particular interest in the 'quenching' ability of eugenol. Three groups of human subjects were studied, and the guinea-pig ear was also used as a model of NIICR. Cinnamic aldehyde, benzoic acid and sorbic acid were all able to produce NIICR in the majority of subjects studied. There was a strong correlation between the susceptibility of each subject to each urticant, but no correlation between the susceptibility to NIICR and age, atopic status or tanning ability. Eugenol caused a reduction in NIICR induced by all three urticants. This 'quenching' effect was apparent even when the eugenol was applied up to 60 min prior to application of cinnamic aldehyde, and its effect was not eliminated by washing. In the guinea-pig-ear model, ear thickening was induced by all three urticants, and this response was inhibited by eugenol.

Contact urticaria is a skin reaction commonly characterized by a weal and flare response occurring on intact skin within a few minutes to an hour after contact with certain rapidly absorbed agents.[1] Minimal reactions may show as erythema with no oedema, and the onset of the reaction may, uncommonly, be delayed for up to 6 h. Depending on the aetiology of the reaction, contact urticaria can be divided into two types: immunological, an IgE-mediated response which only occurs on the skin of subjects who have been previously sensitized to the offending substance; and non-immunological, a non-IgE-mediated response which occurs without prior sensitization. Reactions of the latter type are termed non-immune contact urticaria or more correctly, non-immune immediate contact reactions (NIICR).[2] The mechanism by which substances produce NIICR is not known, but the agents include cinnamic aldehyde, benzoic acid and sorbic acid. Cinnamic aldehyde can also produce a type IV delayed

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hypersensitivity contact reaction in previously sensitized individuals. In contrast, benzoic and sorbic acids rarely produce contact allergy.

Quenching is a term used to describe the phenomenon when a chemical reduces the potential of a fragrance or other material to sensitize. The same term has been used to describe the diminution of NIICR. Eugenol has been reported as quenching delayed hypersensitivity reactions to cinnamic aldehyde,[3] and also as reducing NIICR induced by cinnamic aldehyde.[4,5]

This paper describes investigations using three groups of human subjects to assess the nature of NIICR induced by a number of materials, and to determine the quenching by eugenol.

METHODS

Materials

The fragrance mix and the individual components were obtained from Hermal–Trolab (Denmark). Cinnamic aldehyde, prequenched cinnamic aldehyde and eugenol were supplied by Quest International, Ashford, Kent. Benzoic acid and sorbic acid were obtained from Sigma, Poole, Dorset, U.K. All were made up in petrolatum unless otherwise stated and applied to subjects under occlusion using 11-mm Finn chambers (Epitest Oy, Finland).

Subjects

Three panels of subjects were employed. The details of the techniques used were optimized as experience was gained with each panel.

Panel A. Twenty subjects who had previously demonstrated delayed hypersensitivity reactions to the fragrance mix were selected and a further eight consecutive patients were added with no history of perfume allergy and who were attending the clinic for routine patch tests. The nature of the investigation and its possible relevance to their skin problem was explained and all completed a questionnaire to determine their atopic status. Blood samples were taken, for testing for total IgE, RAST for specific IgE antibody to grass pollens, house dust mite and *Aspergillus fumigatus*, total and differential white blood cell counts, and histamine release from basophils induced by anti-IgE and IgG_4 antibodies. No patient was on antihistamines or steroidal or non-steroidal anti-inflammatory drugs during the tests.

Delayed hypersensitivity was tested by applying the fragrance mix (16%) and its individual ingredients (at 2% each) to each subject's upper back for 48 h. The reactions were read approximately 1 h after removal and at 96 h using the ICDRG scale.

Immediate contact reactions were also assessed on the volar aspect of the forearms to the fragrance mix (16%) and its separate components (2%) at the same time as conventional tests on the back. Test sites were occluded for 40 min using 11-mm Finn Chambers (Epitest Oy, Finland) and on removal of the patches the sites were wiped gently and reactions recorded immediately using a scale of R1–R3 for erythema and U for an urticarial weal. The sites were also inspected 24 and 96 h later, and any type IV allergic reactions noted.

Panel B. Forty-one consecutive patients in the Contact Clinic were tested on the forearm for their immediate contact responses to cinnamic aldehyde (2%), eugenol (2%) and to a cinnamic aldehyde/eugenol mixture (2% of each) with a covered contact time of 30 min. The sites were read 30 min after patch removal using the scale R1–R3 as above. In one patient eugenol was applied to the skin and then cinnamic aldehyde put on the same sites at various times afterwards.

Panel C. Eighty housewives were recruited by questionnaire and were asked whether they would take part in a study to investigate immediate reactions to a number of spices and flavours. Those with a known allergy to perfumes were not included and those who participated in the study were asked to sign an informed consent statement.

Each test material (50 μl) was applied under occlusion to the volar aspect of the forearms of the volunteers. All materials were made up at a concentration of 2% except prequenched cinnamic aldehyde which was prepared at 4% (equivalent to 2% cinnamic aldehyde). The substances were applied, four or five per arm, and left for 20 min. The site of application of the materials was constant in each volunteer, except that the order on each arm for those attending in the afternoon was reversed (the morning: afternoon attendance was 40:40). After removal of the patches, the skin sites were gently wiped once with a tissue. Erythema was scored 10 min later using a scale of R1–R4, with + to indicate intermediate reactions. The presence of oedema and sensations felt by the panellists were also recorded.

Guinea-pigs. In these studies, previously untreated Dunkin–Hartley guinea-pigs of 300–400 g body weight were used.[6] All the test materials were made up in 96% ethanol and four drops (approximately 75 μl) were applied and gently spread over the ears of groups of the guinea-pigs. The thickness of the ears was measured in two places with a Mitutoyo thickness gauge prior to, and at intervals of 30, 60, 90 and 120 min after application of the materials.

In the first experiment, each urticant at 10% in 96% ethanol was applied to the left ears of groups of 10 animals, and the urticant plus eugenol at 10% to the right ears and readings of ear thickness taken. In a second experiment, eugenol at 5% in a 70:30 mixture of acetone: polyethylene glycol 400 was applied 120, 60, 30 or 15 min prior to, or 30 min after the application of 5% cinnamic aldehyde to groups of eight guinea-pigs. Readings were taken before and after the application of the cinnamic aldehyde. The two readings obtained for each animal at each time interval were averaged to give a single reading. The increase in ear thickness was calculated by subtracting the ear thickness prior to application from the ear thickness at each time interval. The maximum increase in ear thickness for each animal was then determined.

Analytical methods

Total IgE was determined using the Phadebas PRIST system (Pharmacia), and total IgG4 by single radial immunodiffusion using antiserum supplied by Seward Immunostics. Specific IgE to grass pollen, house dust mite, *D. pteronysinnus* and *A. fumigatus* were determined using the Phadebas RAST system (Pharmacia).

Histamine release from whole blood was investigated using a procedure similar to that previously described.[7] Blood (6 ml) from each patient was heparinized (150 U) and diluted with 1·5 ml Tris ACM medium containing human serum albumin (0·3 w/v). Aliquots of blood (0·5 ml) were added to 0·5 ml aliquots of anti-IgE and anti-IgG_4 serum (diluted to 1:20 and 1:40 final concentration) or 0·5 ml Tris ACM alone (spontaneous release), and incubated at 37°C for 60 min. The total histamine content was determined by the addition of 0·5 ml blood to 0·5 ml Triton X-100 (1 mg/ml final concentration) in Tris ACM. All tubes were centrifuged at 1500 ***g*** for 30 min at 4°C, and the supernatant removed and stored frozen. Histamine was determined using an automated fluorometric method based on those previously described.[8,9] The results were corrected for spontaneous release (unless otherwise stated) and expressed as a percentage of the total histamine content.

RESULTS

Panel A

Delayed hypersensitivity reactions. Of the 20 patients who had previously shown a reaction to the fragrance mix, only 10 produced delayed hypersensitivity reactions sustained to 96 h in this study when the fragrance mix was applied to the back (Table 1). Seven of these individuals also gave delayed reactions to cinnamic aldehyde, five to cinnamic alcohol, four to isoeugenol, three to hydroxycitronellal, two to geraniol, one to eugenol and one to oak moss absolute. Six subjects also developed delayed hypersensitivity reactions on the forearm where the fragrance mix was applied under occlusion for only 40 min. Four of these also reacted to cinnamic aldehyde, three to cinnamic alcohol, one to isoeugenol and one to hydroxycitronellal. None of the eight patients who had no history of perfume allergy reacted to the fragrance mix or any of the ingredients.

Immediate reactions. In the 10 confirmed fragrance-allergic subjects, cinnamic aldehyde produced an urticarial weal in one and noticeable erythema (R2 and R3) in four, whereas the fragrance mix and other fragrance chemicals produced only a very slight pinkness (R1). In the 18 non-allergic subjects (Table 2), cinnamic aldehyde produced an immediate urticarial reaction in eight and significant erythema in four. The fragrance mix caused an urticarial reaction in two, cinnamic alcohol caused erythema in two and geraniol caused erythema in one subject.

TABLE 1. Delayed hypersensitivity and immediate contact reactions in fragrance allergic subjects (Panel A)

	Patient number										
	2	5	10	18	3	7	11	14	19	20	Total
Fragrance mix 16%	+ + + R1*	+ + + *	+ + + *	+ + + R1*	+ +	+ + *	+ + R1*	+	+	+	10 6*
Cinnamic aldehyde 2%	+ + + R2*	+ + + R1*	+ + *	R1	+ + UR2	+ R3	+ + R3*	+ R2		R1	7 4*
Cinnamic alcohol 2%	+ + + *	+ + + *	+ + *	R1		R1	+ + R1	R1	+ R1		5 3*
Isoeugenol 2%		+ +	+ + + *					+		+	4 1*
Hydroxycitronellal 2%	+ +		+	+ + *							3 1*
Geraniol 2%		+ +	+ +	R1			R1				2
Eugenol 2%			+ +								1
Oak moss absolute 2%						+ +					1
Amylcinnamic aldehyde 2%											0
Petrolatum											0

Delayed hypersensitivity reactions to the fragrance mix and its ingredients were assessed by 48-h occluded patch challenge on the back. Reactions were scored immediately and at 96 h using the ICDRG scale (± to + + +).

Immediate contact reactions were assessed by a 40 min occluded patch on the volar aspect of the forearm, and were scored immediately after patch removal (R, erythema 1–3; U, urticarial weal), and again 24 and 96 h later for signs of a delayed reaction(*).

TABLE 2. Immediate contact reactions in fragrance non-allergic subjects (Panel A)

	Patient number																	
	6	1	8	16	4	9	12	13	17	15	21	22	23	24	25	26	27	28
Fragrance mix 16%		UR3							R1	UR1					R1			
Cinnamic aldehyde 2%	UR3	UR3	R2	UR1	R1	R1	UR2	UR3	R2	UR3	R1	R1	R1	UR2	UR2	R1	R2	R2
Cinnamic alcohol 2%	R2		R1	R1	R1	R1				R1					R1	R1	R2	
Hydroxycitronellal 2%									R1						R1			
Geraniol 2%	R2	R1			R1										R1			

Immediate contact reactions to the fragrance mix and its ingredients were assessed by a 40 min occluded patch on the volar aspect of the forearm. Reactions were scored immediately after patch removal (R, erythema 1–3; U, urticarial weal).

Only fragrances showing a reaction are included.

TABLE 3. Relationship between presence of allergy and development of immediate reactions to fragrance materials

	Allergic subjects			Non-allergic subjects		
Fragrance	Number of reactions	Total number	Ratio	Number of reactions	Total number	Ratio
Cinnamic aldehyde						
Urticarial reactions	1	7	0·14	8	21	0·38
All reactions	6	7	0·86	20	21	0·95
Cinnamic alcohol	2	5	0·4	12	23	0·48
Fragrance mix	3	10	0·3	4	10	0·4
Geraniol	0	2	0	6	26	0·23
Hydroxycitronellal	0	3	0	2	25	0·08

Immediate contact reactions to the fragrance mix and its components were assessed by a 40 min occluded patch on the volar aspect of the forearm. Reactions were scored immediately after patch removal. The ratio indicates the fraction of the subjects displaying an immediate skin reaction.

There was no relationship between NIICR and the presence of delayed hypersensitivity to any of the fragrances (Table 3).

Relationship of immediate responses to atopic and histamine release status. Histamine release induced by anti-IgE antibody from basophils in blood taken from the subjects showed a significant correlation with their total serum IgE level ($r = 0{\cdot}48$), but this was not the case with anti-IgG4. Seven subjects had IgE levels above 200 IU/ml and their completed questionnaires confirmed, together with their RAST estimations, that they could be considered atopics. There was, however, no qualitative or quantitative relationship between the NIICR of atopics and of non-atopics, or with the actual level of IgE found.

Panel B

Immediate reactions to cinnamic aldehyde, eugenol and cinnamic aldehyde/eugenol mixture. Of the individuals tested, 23/41 (55%) reacted to cinnamic aldehyde to some degree (Table 4). In

TABLE 4. Immediate reactions to cinnamic aldehyde, and cinnamic aldehyde eugenol mixture

No.	Sex	Age	CA	CA/E	CA/E cf. CA
1	F	43	R1	R1	=
2	F	31	UR1	UR1	↓*
3	F	38	R2	R2	=
4	F	50	R1	R1	=
5	F	26	UR1	UR1	↓*
6	F	19	R2	R2	=
7	F	39	R2	R2	=
8	F	35	UR2	UR2	↓*
9	F	18	R1	R1	=
10	F	37	R1	R1	=
11	F	75	R2	R2	↓*
12	F	26	R1	R1	=
13	F	35	R2	R2	↓*
14	F	28	UR1	UR1	=
15	F	30	UR1	UR1	=
16	M	72	R2	R2	=
17	M	28	R1	R1	=
18	M	53	UR1	R1	↓
19	F	5	UR2	UR2	=
20	M	28	U	U	=
21	F	32	U	—	↓
22	F	12	R2	—	↓
23*	F	25	UR2	R1	↓

Immediate contact reactions to a mixture of cinnamic aldehyde/eugenol (CA/E) and cinnamic aldehyde alone (CA) were assessed by a 30 min occluded patch on the volar aspect of the forearm. Reactions were scored 30 min after patch removal (R, erythema 1–3; U, urticarial weal).

=, indicates that the reactions to cinnamic aldehyde/eugenol were equal to those to cinnamic aldehyde alone.

↓, indicates that the reaction to cinnamic aldehyde/eugenol was lower than that to cinnamic aldehyde alone.

* Although the scores for the reactions were identical, visual comparison of the two sites indicated that reactions to cinnamic aldehyde/eugenol were lower than those to cinnamic aldehyde alone.

10 the reaction was urticarial, in seven it was erythematous (R2) and in six there was slight pinkness (R1). Of these, 40% (9/23) showed quenching of the reaction when eugenol was combined with cinnamic aldehyde. No reactions were seen to eugenol in any subject.

Pretreatment of the skin with 2% eugenol. On the forearm of one non-allergic subject, 2% cinnamic aldehyde was tested for 15 min occlusion on untreated skin and on sites where 2% eugenol in petrolatum and petrolatum alone had been applied at various times from 8 h to 5 min before. When eugenol or petrolatum was put on to the skin between 8 and 1·5 h preceding the immediate patch test to cinnamic aldehyde, they had no influence on the reaction. When they were applied 5–75 min before the immediate test, however, there was an inhibition of the reaction on treated compared to normal skin. This inhibition on the petrolatum pretreatment site was slight, and was ascribed to the petrolatum acting as a slight barrier to penetration of cinnamic aldehyde. The quenching of the cinnamic aldehyde response on the eugenol-treated sites was greater and was consistent.

In a second experiment a week later, eugenol (2%) and control petrolatum were applied to sites on both arms for 60, 30, 15 and 5 min, then washed off the sites on the left arm only. Immediately 2% cinnamic aldehyde was applied for 15 min to all sites. Eugenol in contact with the skin for only 5 min before being washed off, failed to diminish the NIICR to cinnamic aldehyde. However, prior contact with skin for 15–60 min followed by washing produced some degree of quenching of the immediate reaction, but far less than on sites where it had not been washed off.

Panel C

Cinnamic aldehyde, benzoic acid and sorbic acid all produced erythematous reactions in most of the subjects (Table 5). The mean reaction scores for these materials differed significantly from the mean score for petrolatum (all $P > 0{\cdot}001$). Cinnamic aldehyde produced the strongest reactions, with oedema in 16 subjects, and sensations (mostly described as itching) in eight subjects. Benzoic acid produced slightly less intense reactions, although oedema still occurred in 15 individuals and sensations in 17. Sorbic acid produced only mild erythema in most of the individuals with no oedema and only mild itching in one subject.

Eugenol inhibited the formation of erythema caused by cinnamic aldehyde, benzoic acid and sorbic acid and in all cases this was significant ($P < 0{\cdot}001$, Table 5). The reaction to cinnamic aldehyde was reduced by eugenol in 56 subjects, remained the same in 19 subjects and was increased in five subjects. The corresponding figures for benzoic acid were 71 reduced, nine the same and five increased and for sorbic acid, 59 reduced, 16 the same and five increased. In addition, reactions to prequenched cinnamic aldehyde were less intense than those to cinnamic aldehyde alone in 61 subjects, the same in 12 and were higher in seven subjects. The mean score for prequenched cinnamic aldehyde was significantly less than that for cinnamic aldehyde alone ($P < 0{\cdot}001$).

Of the five subjects whose reactions to cinnamic aldehyde were increased by the presence of eugenol, four also gave a stronger reaction to prequenched cinnamic aldehyde compared to cinnamic aldehyde alone. Correlations seen between reaction intensities produced by cinnamic aldehyde and benzoic acid ($r = 0{\cdot}518$), cinnamic aldehyde and sorbic acid ($r = 0{\cdot}398$) and benzoic acid and sorbic acid ($r = 0{\cdot}418$) were in all cases statistically significant ($P < 0{\cdot}001$).

No correlations were found between the intensity of reactions of any of the urticants and age, tanning ability or atopic status.

Guinea-pigs

All three urticants produced an increase in ear thickness which was maximal at around 30–60 min (Table 6). The addition of eugenol reduced the maximum level of increase caused by each urticant, in each case by around 50%. The reduction in the increase in ear thickness was significant for cinnamic aldehyde ($P < 0{\cdot}001$) and benzoic acid ($P < 0{\cdot}05$), but not for sorbic acid where the response was more variable.

TABLE 5. Reactions to various contact urticants in the presence and absence of eugenol in 80 female volunteers following 20 min occluded application

		Number of subjects showing reactions scored								Number also showing	
Test material	Mean + SEM	R +	R1	R1 +	R2	R2 +	R3	R3 +	R4	Oedema	Sensations*
Cinnamic aldehyde	2·86 ± 0·08	0	2	3	10	17	26	14	8	16	8
Cinnamic aldehyde + eugenol	2·34 ± 0·06†	0	8	26	24	18	1	0	0	3	11
Cinnamic aldehyde + prequenched	2·24 ± 0·06†	0	2	7	40	14	14	2	1	9	10
Benzoic acid	2·74 ± 0·09	0	6	13	9	26	16	6	0	15	17
Benzoic acid + eugenol	1·59 ± 0·06‡	4	21	28	18	7	3	0	0	0	3
Sorbic acid	1·88 ± 0·09	3	18	15	20	9	14	1	0	1	4
Sorbic acid + eugenol	1·19 ± 0·05§	10	39	23	7	1	0	0	0	6	3
Eugenol	1·30 ± 0·04	2	33	39	6	0	0	0	0	0	1
White petrolatum	1·16 ± 0·03	1	53	26	0	0	0	0	0	0	1

All materials were prepared in white petrolatum at a concentration of 2%, except prequenched cinnamic aldehyde which was prepared at 4% (final cinnamic aldehyde concentration = 2%). 50 μl of each test material was applied under occlusion to the volar forearm of 80 female subjects for 20 min. Reactions were scored 10 min after removal of the patches.

* Sensations include itching, tingling, stinging, burning, prickly, smarting, irritation and 'feels warm'.

† Indicates that the mean value was significantly less than that for cinnamic aldehyde alone ($P < 0{\cdot}001$).

‡ Indicates that the mean value was significantly less than that for benzoic acid alone ($P < 0{\cdot}001$).

§ Indicates that the mean value was significantly less than that for sorbic acid alone ($P < 0{\cdot}001$).

TABLE 6. Mean increases in guinea-pig ear thickness (μm) induced by cinnamic aldehyde, benzoic acid and sorbic acid in the presence and absence of eugenol

Material	Increase in ear thickness at time (min) 30	60	90	120	Maximum increase
Cinnamic aldehyde	217·42 ± 20·32*	141·22 ± 13·71	101·6 ± 12·19	22·35 ± 10·66	217·42 ± 20·32
Cinnamic aldehyde + eugenol	109·47 ± 16·00	54·10 ± 10·92	39·62 ± 8·89	31·75 ± 7·62	109·47 ± 16·02
Benzoic acid	158·75 ± 34·04	181·10 ± 27·43	100·07 ± 16·76	134·87 ± 27·94	195·32 ± 28·70
Benzoic acid + eugenol	71·37 ± 25·14	74·67 ± 9·14	55·62 ± 13·97	74·67 ± 11·68	109·47 ± 14·22
Sorbic acid	55·62 ± 23·36	96·77 ± 30·48	69·85 ± 24·13	57·15 ± 12·19	119·12 ± 29·21
Sorbic acid + eugenol	38·1 ± 27·17	33·27 ± 30·98	12·70 ± 30·22	22·35 ± 33·27	54·10 ± 28·95

Materials were prepared in 96% ethanol at 10%. Four drops were applied to the ears of groups of 10 guinea-pigs, and thickness readings taken prior to and at intervals after application as shown. *Values shown are means (± SEM) of the readings (μm).

The mean maximum increase in ear thickness was calculated from the maximum increase in ear thickness measured in each animal.

The quenching effects of eugenol applied at intervals prior to cinnamic aldehyde are shown in Fig. 1. Eugenol applied 15, 30 or 60 min prior to cinnamic aldehyde produced an almost total inhibition of the response. When eugenol was applied 120 min prior to the cinnamic aldehyde, it produced less, but still marked, inhibition. Eugenol applied 30 min after cinnamic aldehyde caused a small but noticeable reduction in the previously induced oedema.

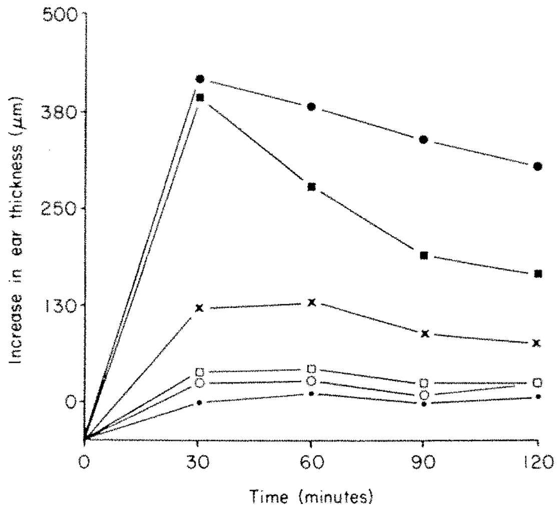

FIGURE 1. The inhibition of cinnamic-aldehyde-induced guinea-pig ear swelling by eugenol. Eugenol (5%) in 70:30 acetone:PEG400 was applied 120, 60, 30 and 15 min prior to, and 30 min after the application of 5% cinnamic aldehyde. The increase in ear thickness was measured at 30-min intervals for 2 h following cinnamic aldehyde application. Each point is the mean ± SEM of readings taken from eight animals. No reaction to 5% eugenol alone was observed. ×, 120 min prior; ○, 60 min prior; □, 30 min prior; ., 15 min prior; ●, no eugenol; ■, 30 min after.

DISCUSSION

In Panel A, which included those with and without proven perfume allergy, 26 of the 28 participants (93%) produced NIICR to 2% cinnamic aldehyde, and confirmed the lack of a relationship between delayed hypersensitivity to perfumes and NIICR. In the second panel of subjects, the incidence of NIICR to 2% cinnamic aldehyde was lower (approximately 55%), but with the refined techniques and timings used for the third, more extensive, group of subjects (Panel C), only two of the 80 subjects (2·5%) failed to produce a more intense reaction to cinnamic aldehyde than to petrolatum alone. Although no previous extensive study on the incidence of NIICR to cinnamic aldehyde has been published, these results tend to confirm the findings of an earlier study[10] in which immediate reactions were observed in all 16 subjects patch tested on the antecubital fossa with 2% cinnamic aldehyde in petrolatum.

In Panel C, reactions to 2% benzoic acid and 2% sorbic acid which were higher than those produced by petrolatum alone were seen in 94% and 66% of subjects, respectively. This level of incidence was somewhat higher than that reported by Lahti,[11] who observed reactions to 5% benzoic acid in 43% and reactions to 2·5% sorbic acid in 15% of the 110 patients studied. Lahti reported that reactions were seen more frequently in male than female subjects. In the present study, only female subjects were used in Panel C. The finding by Lahti that the intensity of the immediate skin reactions did not correlate in any way with the incidence of atopy or age was confirmed by our study. There was also no correlation between the ability to tan and susceptibility to NIICR.

Although 26 of the 28 subjects in Panel A produced NIICR to 2% cinnamic aldehyde, some of the reactions being urticarial in nature, the fragrance mix which also contains 2% cinnamic aldehyde produced reactions in only seven subjects, mostly mild erythema. This suggested that one or more of the other components in the mix was inhibiting the immediate response to cinnamic aldehyde. Since eugenol has been shown to inhibit or 'quench' skin reactions to cinnamic aldehyde,[4] it was suspected that this ingredient may have been at least partly responsible for the diminution. This suspicion was confirmed in the study carried out in Panel B where reactions to cinnamic aldehyde/eugenol were diminished compared to those to cinnamic aldehyde alone in 40% of the subjects who reacted. Studies in the third extensive panel of subjects gave further confirmation, where the scoring system and numbers of subjects were such as to allow statistical analysis. The results revealed that reactions to cinnamic aldehyde/eugenol were significantly lower than those to cinnamic aldehyde alone. Similarly, reactions to prequenched cinnamic aldehyde (a mixture of flavour ingredients consisting of cinnamic aldehyde, eugenol and limonene in the proportions 10 : 7 : 3 used by the perfume industry) were significantly lower than those to cinnamic aldehyde. However, Susskind and Majeti[12] failed to find any evidence of physicochemical interaction between cinnamic aldehyde and eugenol.

The quenching effect of eugenol was also shown to be apparent in one subject even when applied up to 60 min prior to the cinnamic aldehyde, and its effect was not eliminated by washing, unless this was done within 5 min of application. This protracted action of eugenol was also noted in the guinea-pig ear, where eugenol caused a diminution of the swelling response even when applied up to 2 h prior to cinnamic aldehyde.

Although no specific attempt was made in this study to investigate the mechanisms involved in the production of NIICR, a number of points emerged. In the study on Panel C, subjects produced reactions to all three urticants though to widely different degrees. This suggests a broad range of susceptibility to NIICR which did not correlate with age, atopic status or tanning ability. However, susceptibility to each contact urticant correlated well with the other two urticants tested, and this may suggest a common mode of action.

The pattern of quenching seen with eugenol may also throw some light on the mechanisms behind NIICR. In this study, eugenol inhibited reactions to all three urticants, both in humans and guinea-pigs. The mechanism whereby eugenol produces this quenching is unknown, and may be of a non-specific nature (e.g. local vasconstriction). It does not appear to involve the release of histamine, as the pure H_1 antagonist terfenadine is without effect on NIICR.[13] Eugenol has been shown to have an inhibitory action on the cyclo-oxygenase pathway of arachidonic acid metabolism,[14] and may thus inhibit prostaglandin production. It has been suggested that this action is responsible for its quenching action on NIICR.[15] Indomethacin, which is also known to inhibit cyclo-oxygenase, has similarly been shown when administered orally, to inhibit reactions to benzoic acid,[16] but not to cinnamic aldehyde.[17]

This pattern of inhibition has been seen in guinea-pigs in our laboratory. Lahti *et al.*[18], however, using a similar guinea-pig model of NIICR showed that indomethacin inhibited reactions to cinnamic aldehyde, but not to benzoic acid. Other non-steroidal anti-inflammatory drugs applied topically have been shown to inhibit NIICR to subsequent applications of both benzoic acid and cinnamic aldehyde.[19] The inhibition was thought to be due in part to percutaneous absorption and a systemic effect. These results tend to suggest different mechanisms of action of benzoic acid and cinnamic aldehyde.

UV-irradiation, which can cause a reduction in cell-mediated immune responses in the skin by both local and systemic mechanisms, can also produce a long-lasting dose-dependent inhibition of NIICR induced by benzoic acid.[20] Whilst the time course of this effect is similar to the time required for repopulation of the epidermis by Langerhans cells following UV-induced depletion, there is no other evidence to connect these events, and the mechanism for the effect of UVA and UVB on NIICR is unknown.

Although tentative suggestions can be made, the mechanisms involved in producing NIICR require further investigation. The pattern of reactions produced in the guinea-pig ear model of NIICR closely resembles that observed in humans and further studies with this model may provide the key to elucidating NIICR.

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Cutaneous sodium lauryl sulphate irritation potential: age and regional variability

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SUMMARY

To investigate differences in response to irritation according to age and site seven young-adult and eight elderly females were exposed to 0·25% sodium lauryl sulphate (SLS) under patch-test occlusion for 24 h. Ten anatomical regions were tested: forehead, upper arm, volar and dorsal forearm, postauricular, palm, abdomen, upper back, thigh, and ankle. The skin responses were evaluated 24 h post-patch removal by visual scoring and by transepidermal water loss measurements (TEWL). Non-treated symmetrical anatomical regions served as controls. SLS induced a mild erythematous reaction on most anatomical regions except the palm and TEWL was significantly increased as compared with controls. The elderly group demonstrated significantly less susceptibility to SLS-induced irritation for most regions of the body as indicated by visual scores and TEWL measurements. In addition, evaluation of the stratum corneum water content following SLS irritation demonstrated lower responses in the old age group for most regions. The thigh had the highest reactivity and the palm the lowest, in both age groups. These data suggest that young adult skin is more sensitive to SLS than old skin and that SLS irritation varies considerably with respect to region. Moreover, objective TEWL measurements seem to be a better indicator of irritant susceptibility, especially in the elderly, than clinical evaluation by visual scoring.

Individual susceptibility to cutaneous irritation varies considerably depending on a number of exogenous and endogenous factors.[1–3] In recent years, anionic surfactants such as sodium lauryl sulphate (SLS) under patch-test occlusion for 24–48 h has been the main experimental model for cutaneous irritation on human skin.[4] Transepidermal water loss (TEWL) measurements have been used to assess the integrity of the stratum corneum barrier function and to quantify SLS-induced skin damage.[5–7]

Recent studies have determined sex and racial differences in skin irritation. Black skin has a

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higher susceptibility to SLS and a different modulation of irritant reactions after exposure to different amounts of SLS has been demonstrated by TEWL responses.[8] Similarly, a greater sensitivity to SLS was reported in Hispanic skin than Caucasian skin.[9] Lammintausta *et al.* demonstrated no sex-related changes in reactivity to single patch- or repeated open-SLS exposure.[10] Similar findings were observed by Tupker *et al.*[6] No general tendency to stronger skin reactions between the sexes was found using 11 irritants on patients with hand eczema and normal controls.[11]

Studies of irritant responses related to age differences, however, are scarce and conflicting.[12–17] Thus, the present study investigated age and regional variability in SLS-induced irritation using objective TEWL measurements and visual scoring. We also evaluated the stratum corneum hydration state in response to SLS irritation.

METHODS

Study population

Fifteen Caucasian women participated in the study after giving written consent. The group consisted of seven young adult (25·9 ± 1·4 years, mean ± SEM) and eight elderly (74·6 ± 1·9 years) women with no apparent signs of skin disease. There was no history of atopy. The study was approved by the University of California Committee on Human Research.

Applications

Four-hundred microlitres of 0·25% sodium lauryl sulphate (Duponol C, DuPont, Wilmington, DE, U.S.A.) dissolved in distilled water was applied to several regions of the body using occlusive polypropylene chambers (Hilltop Laboratory, Cincinnati, OH, U.S.A.) fixed with non-occlusive paper tape (Scanpore, Norgeplaster, Oslo, Norway). The patches were kept on for 24 h on the following regions: forehead, upper arm (inner middle third), volar and dorsal forearm (centre between wrist and elbow), postauricular, palm (thenar aspect), abdomen (2·5 cm above umbilicus), upper back (scapula), thigh (anterior, upper third), and ankle (medial malleoli). Non-treated, symmetrical anatomical regions served as controls.

Measurements

Irritation was evaluated 24 h post-patch removal to minimize the effect of occlusion on the measurements. The response to irritation was quantified by visual scoring as follows: 0, no erythema; 0·5, equivocal; 1·0, slight, spotty erythema; 2·0, uniform erythema, mild to moderate; 3·0, intense erythema and 4·0, fiery red; and by measurements of transepidermal water loss using an Evaporimeter (EPI ServoMed, Stockholm, Sweden).[18] The probe was held in place perpendicularly to the skin surface until a stable TEWL was established (for approximately 30 s). The skin temperature was measured using a thermistor (Tele-Thermistor, Yellow Springs Instruments, Yellow Springs, OH, U.S.A.) taped onto the skin surface. TEWL values were corrected to a standard skin reference temperature of 30°C as described by Mathias *et al.*[19] The relative humidity (40–49%) and room temperature (20–24°C) were uncontrolled but were recorded. Relative humidity fluctuations were too small compared to variability measurements for detection of a correlation.

Capacitance (CAP) as a measure for stratum corneum hydration was measured in duplicate using a capacitometer (Corneometer SM 820 PC, Courage & Khazaka, Cologne, F.R.G.).[20] This device works on the principle of capacity ratio of the measuring pad due to the high factor of constancy of electricity in water. A standard pressure of 3·5 N was applied . Values of actual measurements were registered about 3 s after the probe was applied to the skin.

Statistics

Differences in TEWL and CAP measurements between control and SLS-treated sites were tested for significance using two-tailed Student's *t*-test for paired samples.[21] Differences between age groups were calculated with two-tailed *t*-test for unpaired samples.[21]

RESULTS

Visual scoring

Visual scores are shown in Table 1. A variation in response to SLS irritation due to age was evident. The elderly group had a weaker irritation response compared to the young reflected by both mean scores and per cent responders. The old age group showed the highest response on the upper arm and postauricular, followed by the forehead. A lower response rate was noted on the volar forearm, upper back and abdomen. Several regions including the thigh, dorsal forearm, ankle and palm demonstrated no erythematous changes. While any irritant response on the palm may show no visible erythema, in a few volunteers palmar reactions were characterized by superficial maceration. In contrast, in the young age group, erythema developed on all the anatomical regions tested except for the palm, with the highest scores for the thigh.

Transepidermal water loss

Although baseline TEWL was not significantly different on most regions between the age groups, it tended to be lower in the elderly (Table 2). The application of SLS significantly increased TEWL on most sites except for the palm and the ankle. On SLS-treated sites, TEWL was significantly lower in the elderly than in the young age group on several regions of the body, namely, the upper arm, dorsal forearm, abdomen, upper back and thigh. The highest increase in TEWL was noted on the thigh while the lowest was noted on the palm.

Capacitance

Baseline CAP did not vary between age groups for all regions except for the upper arm and

TABLE 1. Visual scores: 24 h post-patch removal

	Young		Old		
Region	Mean ± SEM	% Responders	Mean ± SEM	% Responders	Significance*
Forehead	0·50 ± 0·19	57·1	0·44 ± 0·26	37·5	NS
Upper arm	0·86 ± 0·24	85·7	0·38 ± 0·16	50·0	NS
Volar forearm	0·93 ± 0·23	85·7	0·13 ± 0·08	25·0	$P \leqslant 0{\cdot}005$
Dorsal forearm	0·64 ± 0·18	71·4	0·00 ± 0·00	0·0	$P \leqslant 0{\cdot}005$
Postauricular	0·50 ± 0·15	71·4	0·44 ± 0·18	50·0	NS
Palm	0·00 ± 0·00	0·0	0·00 ± 0·00	0·0	NS
Abdomen	0·86 ± 0·26	71·4	0·13 ± 0·08	25·0	$P \leqslant 0{\cdot}02$
Upper back	0·86 ± 0·24	85·7	0·19 ± 0·13	25·0	$P \leqslant 0{\cdot}05$
Thigh	1·36 ± 0·37	100·0	0·00 ± 0·00	0·0	$P \leqslant 0{\cdot}005$
Ankle	0·14 ± 0·09	28·6	0·00 ± 0·00	0·0	NS

* Two-tailed student's *t*-test. NS, not significant ($P > 0{\cdot}05$).

TABLE 2. SLS-irritant patch-test reactions: transepidermal water loss measurements (TEWL, g/m^2/h)

Region	Young	Old	Significance
Forehead			
Control	7·1 ± 1·1	4·8 ± 1·7	NS
SLS	15·8 ± 2·1*	14·1 ± 5·0*	NS
Upper arm			
Control	3·8 ± 0·7	1·8 ± 0·6	$P \leqslant 0{\cdot}05$
SLS	17·0 ± 5·2*	4·6 ± 1·6*	$P \leqslant 0{\cdot}05$
Volar forearm			
Control	5·0 ± 1·4	2·3 ± 0·8	NS
SLS	13·5 ± 4·5	5·3 ± 1·9*	NS
Dorsal forearm			
Control	3·8 ± 0·7	2·1 ± 0·8	NS
SLS	11·6 ± 2·8*	4·2 ± 1·5*	$P \leqslant 0{\cdot}05$
Postauricular			
Control	6·1 ± 0·8	6·6 ± 2·3	NS
SLS	11·4 ± 2·3*	11·6 ± 4·1*	NS
Palm			
Control	31·0 ± 7·7	26·2 ± 9·3	NS
SLS	26·9 ± 4·0	22·5 ± 8·0	NS
Abdomen			
Control	5·4 ± 0·9	1·9 ± 0·7	$P \leqslant 0{\cdot}01$
SLS	20·0 ± 4·7*	5·1 ± 1·8*	$P \leqslant 0{\cdot}01$
Upper back			
Control	5·6 ± 1·2	3·3 ± 1·2	NS
SLS	18·2 ± 3·5*	6·6 ± 2·3*	$P \leqslant 0{\cdot}02$
Thigh			
Control	5·1 ± 1·6	2·2 ± 0·8	NS
SLS	24·6 ± 7·6*	7·1 ± 2·5*	$P \leqslant 0{\cdot}05$
Ankle			
Control	6·2 ± 1·7	2·5 ± 0·9	NS
SLS	6·5 ± 1·8	3·7 ± 1·3	NS

* Differences were compared between control and SLS-treated sites. NS, not significant ($P > 0{\cdot}05$).

postauricular where CAP was significantly higher in the elderly (Table 3). SLS irritation did not influence CAP in the young. However, stratum corneum hydration was decreased after SLS treatment in the elderly for most regions.

DISCUSSION

Sodium lauryl sulphate, an anionic surfactant, is a known cutaneous irritant.[4] Although the exact pathomechanisms of SLS-irritation are unknown, keratin denaturation, emulsification of lamellar lipids in the intercellular spaces of the stratum corneum and chemotaxis and

TABLE 3. SLS-irritant patch-test reactions: capacitance

Region	Young	Old	Significance
Forehead			
Control	97.3 ± 3.5	89.5 ± 7.3	NS
SLS	77.1 ± 10.2	95.9 ± 4.3	NS
Upper arm			
Control	77.3 ± 1.8	92.2 ± 2.9	$P \leqslant 0.001$
SLS	71.4 ± 4.7	71.7 ± 5.7*	NS
Volar forearm			
Control	81.1 ± 3.1	87.9 ± 3.9	NS
SLS	69.9 ± 4.6	81.1 ± 5.2	NS
Dorsal forearm			
Control	73.4 ± 4.0	84.4 ± 5.3	NS
SLS	67.5 ± 5.4	64.3 ± 6.0*	NS
Postauricular			
Control	94.9 ± 4.6	112.7 ± 2.5	$P \leqslant 0.005$
SLS	100.1 ± 3.2	99.3 ± 4.7*	NS
Palm			
Control	86.9 ± 6.4	71.6 ± 10.5	NS
SLS	94.9 ± 7.7	71.8 ± 5.2	$P \leqslant 0.05$
Abdomen			
Control	79.4 ± 4.3	79.2 ± 5.5	NS
SLS	80.2 ± 4.5	66.8 ± 4.1	$P \leqslant 0.05$
Upper back			
Control	103.1 ± 2.7	104.7 ± 3.2	NS
SLS	99.6 ± 4.2	87.9 ± 3.7*	NS
Thigh			
Control	69.2 ± 3.6	74.9 ± 2.8	NS
SLS	80.2 ± 6.7	66.2 ± 4.2	NS
Ankle			
Control	58.1 ± 8.1	72.3 ± 4.7	NS
SLS	45.9 ± 4.4	64.9 ± 3.5	$P \leqslant 0.005$

* Differences were compared between control and SLS-treated sites. NS, not significant ($P > 0.05$).

chemokinesis of neutrophils eliciting an inflammatory response have been demonstrated.[22-24]

In this study, we investigated the influence of age and anatomical site variability on SLS irritation. By visual scoring and TEWL measurements, young adults had a higher irritant response after SLS application than the old. A fivefold difference in relative TEWL for different regions was noted in the young in contrast to a threefold difference in the elderly group. Evidently, the elderly were less susceptible to SLS-irritation. These findings agree with earlier studies on the lower irritancy potential with increasing age.[12-14] Lejman *et al.* found higher irritant responses to SLS in the young than the old.[12] Using a variety of excitants, DMSO, histamine, ethyl-nicotinate, chloroform–methanol and lactic acid, Grove *et al.* demonstrated milder reactions to these substances with age.[13] A decrease in the number of positive reactions to croton oil has been reported with age, but no age dependence was observed for thymoquinone and crotonaldehyde.[14]

Few comparative studies on the response to irritation of different regions of the skin have been done. Magnusson and Hersle demonstrated a significantly stronger reaction to benzalkonium chloride on the upper back than the lower back, forearm or thigh.[15] Britz and Maibach demonstrated a stronger reaction to benzalkonium chloride and maleic acid on vulvar than forearm skin,[16] but no differences between vulvar and forearm skin reactivity were found using the model irritant SLS at various concentrations.[17] In this study, TEWL measurements revealed regional variation in reactivity in response to SLS irritation with the thigh as the most vulnerable site followed by the upper arm, abdomen, upper back, dorsal and volar forearm, postauricular and ankle, with the palm as the least vulnerable location. These findings contrast with those of Magnusson and Hersle[15] who found the back to be more reactive to benzalkonium chloride than the thigh. Nevertheless, susceptibility to SLS irritation with respect to region was comparable between the two age groups. Since cutaneous irritant responses to various irritants might be mediated by distinctly different pathophysiological pathways,[25] regional susceptibility to diverse irritants vary accordingly. Additionally, stratum corneum barrier properties have been associated with stratum corneum lipid composition,[26] regions with higher neutral lipids and lower sphingolipids were generally associated with superior barrier properties.[26]

Several anatomical and physiological changes occur with skin ageing. However, the mechanisms underlying differences in irritant response with ageing remain unknown. The thickness of the stratum corneum remains unchanged as does the number of cell layers.[27] Moreover, the stratum corneum turnover time increases with age which means that an irritant or sensitizing substance remains longer on the skin;[27] a compromised cutaneous blood vessel network with ageing may lead to a decreased inflammatory response, decreased absorption and decreased clearance.[27,28] The penetration of methylnicotinate, testosterone, estradiol, hydrocortisone, benzoic acid, acetylsalicylic acid and caffeine were studied in young and old age groups. Only the penetration of benzoic acid and acetylsalicylic acid was significantly higher in the elderly, suggesting that age-related differences in percutaneous penetration are relatively small.[29] There is also evidence that quantitative differences in lipid content of the stratum corneum are more accurate predictors of regional variations in skin permeability than either stratum corneum thickness or cell number.[30] It would be interesting to know whether similar differences exist accounting for age variability in irritant susceptibility. Finally, the effects of stratum corneum hydration on irritancy potential is probably non-contributory. In this study, baseline CAP did not differ between age groups on most regions. With SLS treatment, CAP was generally lower only in the elderly group, but SLS irritation was generally higher in the young.

In summary, a difference in susceptibility to SLS-induced irritation was found between the two age groups which appeared to be related to the difference in response of the stratum corneum barrier function and to regional variability. These data suggest that young adult skin is more sensitive to SLS than old skin. Furthermore, objective TEWL measurements seem to be a better indicator of irritant susceptibility, especially in the elderly, than clinical evaluation by visual scoring.

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Conjunctival involvement in pemphigus vulgaris: a clinical, histopathological and immunofluorescence study

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SUMMARY

Eleven patients with pemphigus vulgaris and with eye complaints had histopathological and direct immunofluorescence (DIF) studies on biopsies of their conjunctivae. In eight of the patients the ocular symptoms preceded the other manifestations of pemphigus. None of the 11 patients had any detectable conjunctival blisters or erosions. The conjunctivitis in three of the patients proved on histopathology and DIF to be a manifestation of pemphigus vulgaris. In the remaining patients, conjunctival hyperaemia with or without a mucoid discharge was observed, but there were no specific histopathological features, although one of them had a positive DIF.

Conjuctival involvement may occur in pemphigus vulgaris (PV), though the literature regarding the clinical and histopathological features of eye involvement is sparse. The involvement may take the form of blisters and erosions, though this is regarded as an unusual event[1] as are eyelid erosions.[2,3] There is no consensus of opinion as regards conjunctival abnormalities other than blisters, though several textbooks on ophthalmology regard catarrhal conjunctivitis as an almost invariable involvement in PV.[4–6] Neither recent review articles[7,8] nor textbooks of dermatology[9–12] describe this type of involvement in pemphigus and there is little literature on the histopathology and the immunopathological findings of the conjunctiva in PV.

The purpose of this study was to investigate the clinical, histopathological and immunofluorescence findings of the conjunctiva in 11 patients with PV who had eye symptoms.

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METHODS

Patients

Eleven patients (nine females and two males) with PV were studied. They all had eye complaints and were referred for ophthalmological evaluation in our institution. The relevant clinical features and laboratory data are given in Table 1. Eight of the patients had oral lesions, either alone or in addition to skin lesions, and three of them had only cutaneous involvement. All but two were newly diagnosed cases of PV and were receiving no systemic therapy. Patients 7 and 11 were evaluated during a relapse of their PV that had been present for 3 and 5 years, respectively, and who were both on a maintenance dose of prednisone of 20 mg daily.

Conjunctival biopsy

After informed consent had been obtained a biopsy was taken from the infero-temporal bulbar conjunctiva following local anaesthesia with oxybuprocaine drops. Two snip biopsies, 2 mm each, were taken under slit-lamp viewing. One was embedded in paraffin wax and then processed routinely for histopathology and the second biopsy was snap frozen for immunofluorescence (IF) studies. Cryostat sections were cut and stained with fluorescein-conjugated antisera specific for human IgG and C3 (Tago, Burlingame, CA, U.S.A.).

RESULTS

Ocular symptoms and signs

In eight of the patients the eye symptoms preceded the appearance of mouth and skin lesions by several days to months. All the patients complained about irritation and excessive watering of the eyes as well as the sensation of having a foreign body in the eye. On examination, in five of the patients there was a conjunctivitis with hyperaemia and some mucoid discharge. The remaining patient had only conjunctival hyperaemia. In one patient erosions were observed on the lower eyelid margin. There was no evidence of conjunctival blisters, erosions, synechiae or subepithelial fibrosis. There were no signs of epithelial keratopathy or destruction of medial

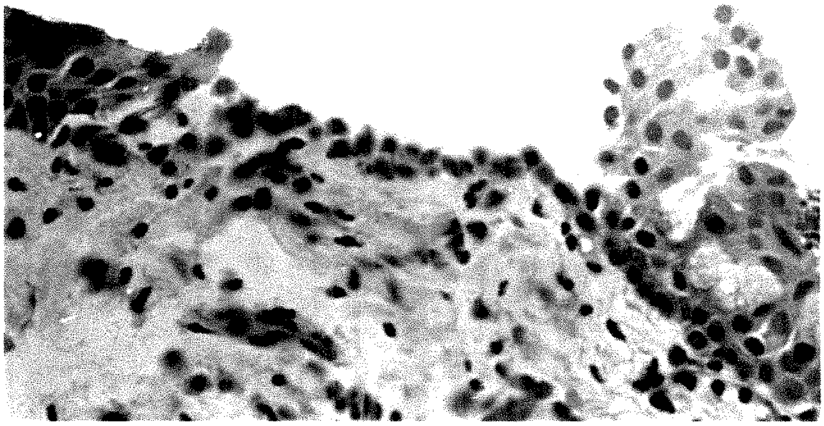

FIGURE 1. Conjunctiva with suprabasal splitting of the epithelium (Haematoxylin and eosin, × 400).

TABLE 1. Symptoms, signs, clinical, histopathological and IF findings of the conjunctiva in patients with pemphigus vulgaris

No.	Age (years)/sex	Site of lesions	Eye complaints	Duration of complaints prior to diagnosis of PV	Clinical appearance of the conjunctiva	Suprabasal acantholysis (positive/negative)	Direct IF
1	61/F	Palpebral skin, mouth	Irritation and watering	9 months	Hyperaemia with mucoid discharge	Not done	Negative
2	62/F	Skin and mouth	Irritation and watering	Few days	Hyperaemia	Negative	Negative
3	65/F	Mouth	Foreign body sensation and watering	3 weeks	Hyperaemia, lower lid margin erosions	Negative	Negative
4	26/F	Skin and mouth	Irritation and watering	Uncertain	Hyperaemia	Negative	Negative
5	51/F	Skin	Irritation	Uncertain	Hyperaemia	Negative	Negative
6	39/F	Skin and mouth	Irritation	2 weeks	Hyperaemia	Negative	IgG, IC*
7	70/M	Skin and mouth	Irritation and watering	Uncertain	Hyperaemia with mucoid discharge	Negative	Negative
8	72/M	Skin	Irritation and watering	2 months	Hyperaemia	Negative	Negative
9	60/F	Skin	Irritation	Few weeks	Hyperaemia with mucoid discharge	Positive	IgG, IC
10	75/F	Mouth, vulva, skin	Irritation and watering	Few days	Hyperaemia with mucoid discharge	Positive	IgG, IC
11	61/F	Mouth and skin	Irritation and watering	Few days	Hyperaemia with mucoid discharge	Positive	IgG, C3 IC

* IC, intercellular

canthal structures. Fluorescein staining of the conjunctivae and cornea gave negative results and the Schirmer test was normal.

Histopathology

On light microscopy the typical appearance of PV with acantholysis and splitting above the basal layer was found in three of the cases that had conjunctival hyperaemia with a mucoid discharge (Fig. 1). A slight subepithelial lymphocytic infiltrate was detected. In the other eight patients the histopathology of the biopsies was either non-specific or negative.

Direct immunofluorescence

Direct IF performed on the biopsies of the conjunctivae revealed in four patients the typical pattern of PV, with intercellular deposits of IgG in the epidermis. In one of the patients there was also seen C3 (Fig. 2). These four patients included three of those who had the typical histopathology of PV. In the other seven patients, DIF on the conjunctival biopsies was negative.

Indirect immunofluorescence

An indirect IF test on normal human skin using the serum of the patients was positive in seven in a titre of 1/20 to 1/640 and was negative in two others. In the remaining two patients this test was not performed. There was no correlation between the indirect IF and the results of the direct IF studies on conjunctivae.

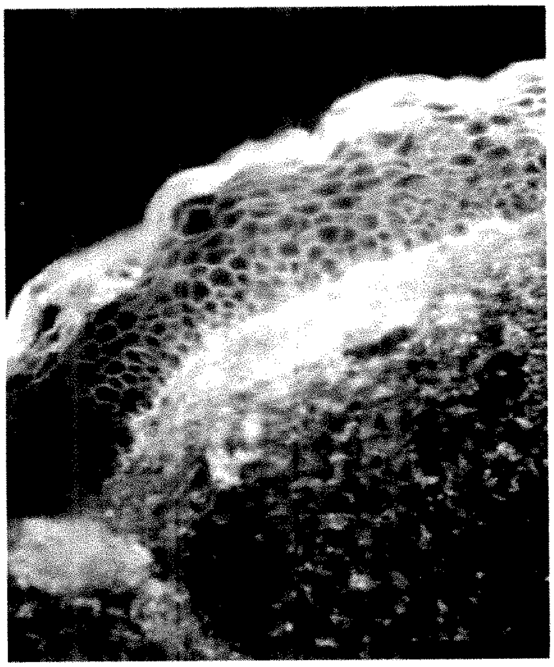

FIGURE 2. Direct immunofluorescence of the conjunctiva showing intercellular deposits of IgG (× 300).

Follow-up

The eye symptoms rapidly improved following either commencement of systemic therapy with prednisone or an increase in the dose in those patients who had relapsed.

DISCUSSION

It is stated in a textbook that in patients with PV 'conjunctival bullae are not common although buccal and pharyngeal lesions are frequent, but catarrhal conjunctivitis is almost invariable'.[4] A catarrhal conjunctivitis is reported as a common finding among patients in Brazil with endemic pemphigus, 'fogo selvagen'.[13] Both of these observations were made in the days before IF techniques were applied for the classification of bullous disorders. The first documented case of PV in which IF studies were carried out on the conjunctiva was in 1975, and intercellular IgG staining was demonstrated.[1] More recently, conjunctival involvement with positive direct IF findings were reported in two patients with pemphigus.[14]

The present study confirms that conjunctival blistering and erosions are rare occurrences in PV, since none of the 11 patients with eye symptoms had any detectable blisters, erosions or synechiae. Of importance is the finding that conjunctivitis is indeed a manifestation of PV as determined by the histopathology and IF findings in three of our 11 patients. In all three patients the ocular symptoms preceded the other manifestations of PV and this indicates that they may be sometimes the earliest sign of the condition. As one of these three patients with conjunctivitis had only cutaneous lesions, it would appear that eye involvement is not necessarily associated with involvement of other mucous membranes. However, it should be stressed that in our study patients were selected for eye symptoms.

In the remaining eight patients, the histopathological findings were either negative or non-specific, although the DIF on the conjunctival biopsy in one was positive. The exact relationship between the conjunctival abnormalities in these eight patients and PV is not clear. In view of the temporal relationship between the appearance of eye symptoms and PV and the parallel regression of both, it would appear that these eye abnormalities may well be related, particularly as non-specific conjunctivitis is a known feature of several immune-mediated diseases.[15]

The low yield of positive DIF findings in the conjunctivae may be due to the low expression of PV antigen. Alternatively, it is possible that the deposition of immunoreactants in the conjunctivae is not uniform, resulting in low sensitivity of DIF studies, as has been reported for ocular cicatricial pemphigoid.[16]

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Effect of long-term PUVA treatment of psoriasis on the collagen and elastin gene expression and growth of skin fibroblasts *in vitro*

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SUMMARY

The proliferation rate, collagen metabolism and collagen and elastin messenger-RNA levels were studied in fibroblasts derived from patients who had received many courses of either systemic 8-methoxypsoralen or topical trioxsalen PUVA treatment. The proliferation rate of fibroblasts as measured by the incorporation of ^{3}H-thymidine or by cellular division was decreased in those obtained from patients who had PUVA treatment as compared with controls. Collagen synthesis was slightly increased in the cells from PUVA-treated patients, but the relative collagen synthesis and the ratio between types I and III collagen were unchanged. The levels of collagen and elastin mRNAs were increased in fibroblasts derived from the PUVA-treated patients. No significant differences in histology or immunochemistry could be found in the biopsies taken from topical and systemic PUVA-treated patients.

PUVA therapy, which is widely used for the treatment of various skin diseases such as psoriasis, vitiligo and lichen planus, has the long-term side-effect of premature ageing of the skin.[1] Distinct histological changes have been observed in the connective tissue after PUVA treatment, and these include the disappearance of elastic fibres at an early stage and later an increased amount of thickened elastin.[1] No marked ultrastructural changes have been noted in the collagen after PUVA treatment, but experimental studies using a nude mouse model and fibroblasts have shown that its metabolism may be affected.[1] PUVA treatment of fibroblasts *in vitro* reduces the proliferation rate,[2] and UV irradiation reduces the activity of prolyl hydroxylase in the nude mouse skin.[3] Prolyl hydroxylase is a key enzyme for the intracellular

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biosynthesis of collagen, in the absence of which no proper collagen can be synthesized.[4] Thus, reduced activity could lead to diminished deposition of collagen *in vitro*.

We studied the proliferation rate of skin fibroblasts derived from patients treated either topically, with trioxsalen in bath water, or systemically, with 8-methoxypsoralen, with PUVA and measured the metabolism of collagen and the levels of collagen and elastin mRNAs in these fibroblasts.

METHODS

The clinical features of 12 patients included in the study are shown in Table 1. Six of the subjects had received topical bath trioxsalen PUVA-treatment as previously described,[5] and six had been treated with systemic oral 8-methoxypsoralen.[6] The mean cumulative UVA-dose was 481 J/cm^2 in the topical PUVA-group and 1460 in the systemic PUVA-group.

Skin biopsies were taken from the uninvolved skin of the patients either from the upper arm or from the back. Control biopsies were taken during surgery on age-matched patients attending the Department of Dermatology at Oulu University Central Hospital.

Portions of the biopsies for light microscopy and immunohistochemical studies were taken from the lesional skin of the patients, and for light microscopy, were fixed in 4% phosphate-buffered formaldehyde and processed routinely. Paraffin-embedded sections were cut at 5 μm and stained with haematoxylin and eosin and Verhoeff van Gieson stains. The sections for immunohistochemistry were deparaffinized and treated with 0·4% pepsin (Sigma Chemical Co., St Louis, MO, U.S.A.) to enhance the availability of the antigens.[7] Endogenous peroxidases were inactivated by exposing the sections to 0·1% hydrogen peroxide, and peroxidase–antiperoxidase staining for type III procollagen and fibronectin were performed

TABLE 1. Clinical data of patients treated with topical or systemic PUVA therapy

Culture code	Diagnosis	Sex	Age (years)	Cumulative UVA dose (J/cm^2)	Site of biopsy
Topical-PUVA					
T1	PV	M	60	305	Upper arm
T2	PV	F	74	304	Abdomen
T3	PV	M	60	857	Upper arm
T4	PV	M	38	343	Upper arm
T5	PA	F	55	527	Upper arm
T6	PV	M	40	550	Upper arm
Systemic-PUVA					
S1	PA	F	46	1913	Back
S2	PV	M	34	1179	Back
S3	PV	F	43	1065	Back
S4	PV	F	42	1210	Back
S5	PV	M	58	2822	Back
S6	PV	M	48	570	Back

PV, psoriasis vulgaris.
PA, psoriasis arthropathica.

according to the method of Sternberger.[8] The antibody to pro-III collagen was donated by Drs J.Risteli and L.Risteli, Oulu. Purification of the antigen and preparation of the antibody were performed as previously described.[9] The concentration of the antibody was 0·31 mg/ml and this was used at a dilution of 1:25. The antifibronectin was obtained from Dako A/S (Copenhagen, Denmark). Normal rabbit serum and phosphate-buffered saline (PBS) were used in place of the primary antibody for the controls.

Primary cell cultures were established by routine methods, and subcultured on plastic culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 50 μg/ml of ascorbate, 290 μg/ml of L-glutamine, penicillin 200 U/ml and streptomycin 100 μg/ml. The analyses of fibroblast cultures were carried out after 4–9 passages.

Collagen biosynthesis

Fibroblasts at confluence were incubated for 24 h in DMEM supplemented as above, but the serum was replaced with 2% dialysed fetal calf serum and [^{14}C]-proline was added. After the labelling period the medium was collected and proteinase inhibitors were added to give final concentrations of 25 mM Na_2 EDTA, 10 mM *N*-ethylmaleimide, 1 mM phenylmethylsulphonyl fluoride and 1 mM para-aminobenzamidine. The proteins in the medium were then precipitated by adding ammonium sulphate. The precipitates were collected by centrifugation for 30 min at 10,000 ***g*** after stirring overnight at 4°C. These were assayed for [^{14}C]-hydroxyproline and total incorporation of [^{14}C] radioactivity by a specific radiochemical method.[10]

The cell layer was rinsed with PBS and the cells scraped with a rubber policeman into 2 ml of 0·4 M NaCl, 0·1 M Tris-HCl at pH 7·5, containing the proteinase inhibitors described above. The cells were sonicated at 60 Hz for 30 s. These samples were used for assays of total cell protein[11] and DNA.[12] For determination of genetically distinct collagen types, parts of the samples were submitted to limited pepsin proteolysis and precipitated with NaCl. The SDS-PAGE was performed using 8% polyacrylamide gels, with and without delayed reduction with 2-mercaptoethanol.[13,14] The collagen polypeptides were visualized by fluorography[15] and the bands representing α1 (III), α1 (I) and α2 (I) were quantified with an automatic computing densitometer.

Assays of enzyme activities

The cells were grown to early confluence and harvested by trypsinization and stored in a pellet form at −70°C for up to 3 weeks. After thawing, they were homogenized in a cold solution containing 0·2 M NaCl, 0·1 M glycine, 0·1% (w/v) Triton X-100, 0·1% (w/v) soya-bean trypsin inhibitor and 0·02 M Tris-HCl buffer at pH 7·5 (3–4×10^6 cells/ml). The homogenates were centrifuged at 15,000 ***g*** for 30 min at 4°C and aliquots of the supernatants were taken for the enzyme assays.

The prolyl 4-hydroxyproline (PH) activity was assayed by measuring the formation of radioactive 4-hydroxylase using a [^{14}C]-proline-labelled type I protocollagen substrate.[16]

For the assay of collagenase activity, the fibroblasts were cultured in serum-free DMEM for 6 h. The medium was collected and aliquots were subjected to brief trypsin proteolysis using 0·1–10 μg trypsin per ml for 10 min at 25°C. The trypsin was then inactivated by adding soya-bean trypsin inhibitor (50 μg/ml), and collagenase activity was assayed by incubating the samples with [^{3}H]-proline-labelled type I collagen, as described previously.[17]

Assay of DNA synthesis

The DNA synthesis was estimated by determining the incorporation of [^{3}H]-thymidine into the trichloracetic acid-precipitable material. The cells were transferred to a 24-well plate, with approximately 2×10^4 cells per well. After 48 h pre-incubation, the cells were labelled with $[^3H]$-thymidine (1 μCi/well) for 4 h. They were then washed thoroughly with PBS and disrupted by incubating for 1 h at 40°C in 1% (w/v) SDS, 1 mM Na_2EDTA and 50 mM Tris-HCl at pH 7·5, supplemented with 1 vol of 20% (w/v) trichloroacetic acid containing 100 μg of calf thymus DNA as a carrier. The precipitates were collected by centrifugation at 15,000 **g** for 20 min, washed twice with 10% (w/v) trichloroacetic acid and dissolved in Lumagel, and the radioactivity counted.

Assay of type I procollagen and elastin mRNA steady-state levels

The total RNA was isolated as previously described[18] and used for a slot-blot hybridization assay.[19] The RNA samples were dotted on to nitrocellulose paper, and the filters were hybridized with recombinant plasmid Hf677 containing cDNA for human pro α1 (I) collagen mRNA,[20] elastin[21] and γ-actin.[22] The recombinant plasmid was labelled with $[^{32}P]$ nucleotides to a specific activity of $5\text{–}8 \times 10^8$ c.p.m./μg by nick translation.[23] The amount of recombinant $[^{32}P]$-labelled plasmid hybridized to mRNA was visualized by autoradiography using Kodak X-Omat film and cassettes with intensifying screens. The autoradiograms were quantified by scanning with a Kontes K 495000 densitometer connected to a Sepctra-Physics SP411 computing integrator.

Other assays

To study the attachment and proliferation of fibroblasts, the same number of fibroblasts (5×10^4) was inoculated into culture wells and the number of cells or the amount of DNA assayed after 24 h and 7 days.

Statistics

Wilcoxon rank-sum test (two-tailed) was used for the statistical analyses.

RESULTS

Histological and immunohistochemical studies

There was a slight to moderate increase in melanocytes in eight of the 10 skin biopsies from the PUVA-treated cases (Fig. 1). There was slight epidermal hyperpigmentation and a minimal solar elastosis in five cases, and there was nuclear atypia with variation of the nuclear size, staining and orientation of both melanocytes and keratinocytes in four of the biopsies. Also necrotic keratinocytes and thickening of the basement membrane zone were seen.

Staining with anti-pro III collagen antibody showed a moderate to strong dermal reaction in the vicinity of the subepidermal basement-membrane zone, and a slight reaction or no staining at all around the blood vessels, and the periadnexal and other dermal areas. The findings were similar in the control specimens. Fibronectin appeared to be evenly distributed in all the specimens. There were no marked differences in the histology or immunochemistry between those biopsies from patients who had been treated with topical or with systemic oral PUVA.

Thymidine incorporation, proliferation rate and attachment of fibroblasts

The $[^3H]$-thymidine uptake was slightly decreased in both the topical PUVA ($P < 0{\cdot}114$) and in fibroblasts from those who had systemic PUVA (Fig. 2a, b). However, when the cells were cultured for 7 days, the increase in the amount of DNA was about the same in control and topical

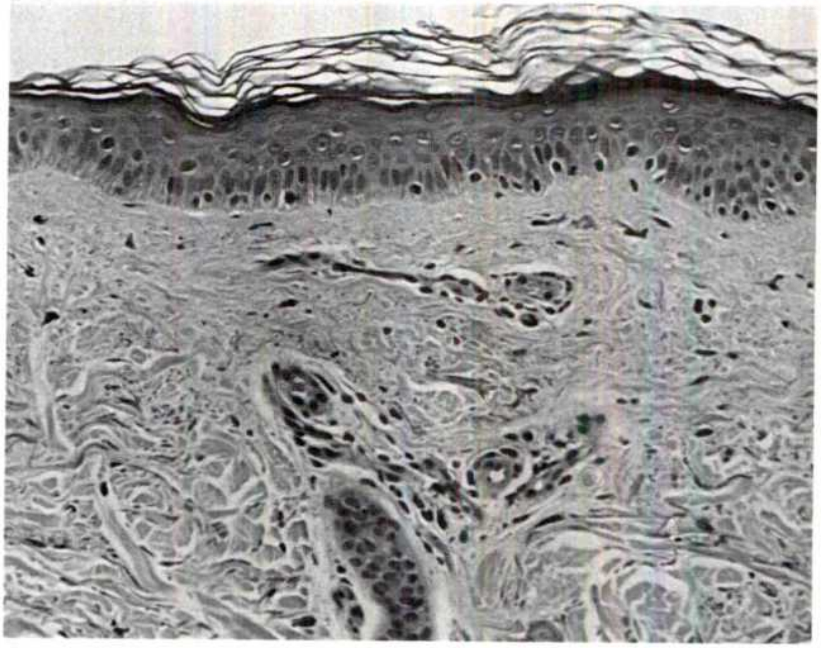

FIGURE 1. Skin biopsy from a systemic PUVA-treated patient (case S 6) showing hyperplasia of the epidermal melanocytes with darkly stained nuclei. Haematoxylin and eosin × 140.

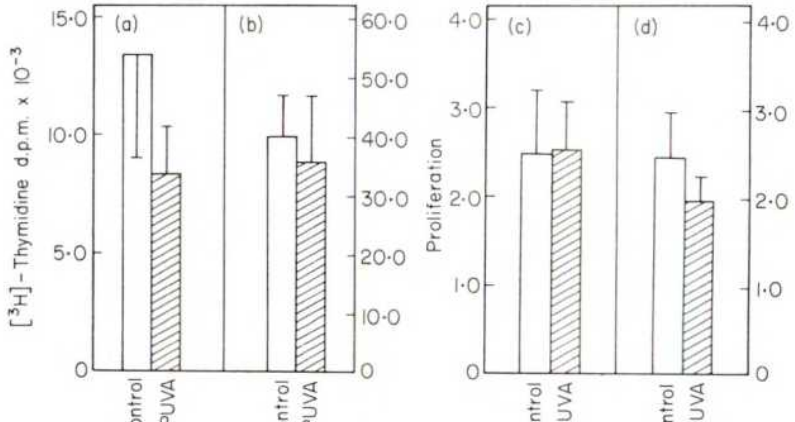

FIGURE 2. [^{3}H]-Thymidine incorporation and proliferation rate in fibroblasts derived from PUVA-treated patients and controls. Control cells and cells derived from PUVA-patients were labelled in two separate experiments (a and b) with [^{3}H]-thymidine as described. The values are the mean ± SD

To measure the proliferation rate (c and d) the DNA was measured on day 1 after inoculation of the cells, and parallel flasks were used for the measurement of DNA on day 7. The values are the ratio of DNA on day 7 to that on day 1 (mean ± SD). Number of cell lines: (a) and (c) controls, $n=4$; T-PUVA, $n=6$. (b) and (d) controls, $n=4$; S-PUVA, $n=3$.

PUVA fibroblasts, and only slightly less in those fibroblasts from patients who had systemic PUVA ($P < 0{\cdot}14$) (Fig. 2c, d).

The relative attachment of fibroblasts at 24 h was 1·0 ± 0·4 in the control cells, 0·6 ± 0·2 in the topical PUVA fibroblasts and 0·8 ± 0·6 in the fibroblasts from patients who had systemic oral PUVA.

Incorporation of [^{14}C]-proline, synthesis of [^{14}C]-hydroxyproline and activity of prolyl hydroxylase

Total protein synthesis as measured in terms of the incorporation of [^{14}C]-proline into the non-dialysable fraction was increased slightly in the PUVA-treated cells (Table 2). Similarly, collagen synthesis, measured in terms of the synthesis of [^{14}C]-hydroxyproline was increased slightly in the PUVA-treated cells (Table 2). The relative synthesis of collagen, expressed as the synthesis of [^{14}C]-hydroxyproline relative to total radioactivity, was similar in both topically

TABLE 2. Total incorporation, synthesis of [^{14}C]-hydroxyproline, collagen types and activity of prolyl hydroxylase in PUVA-treated and control fibroblasts

Cell line	Total incorporation* (d.p.m. × 10^{-3})	[^{14}C]-Hydroxyproline* (d.p.m. × 10^{-3})	Ratio type III/I†	Prolyl hydroxylase‡ d.p.m. × 10^{-2}/μg protein
Experiment 1				
T1	2·00	0·49	0·30	1·83
T2	3·09	0·88	0·12	2·50
T3	6·47	1·55	0·16	2·36
T4	4·05	1·05	0·15	2·39
T5	6·08	1·46	0·13	1·66
T6	4·39	1·15	0·18	2·62
Mean ± SD	4·35 ± 1·71§	1·10 ± 0·39§	0·17 ± 0·07	2·23 ± 0·39
Controls ($n = 4$)				
Mean ± SD	1·81 ± 0·96	0·45 ± 0·25	0·21 ± 0·04	2·00 ± 0·39
Experiment 2				
S1	6·65	1·71	0·32	0·92
S2	8·82	2·33	0·27	2·09
S4	7·10	1·48	0·31	2·67
Mean ± SD	7·52 ± 1·13¶	1·84 ± 0·44¶	0·30 ± 0·02	1·85 ± 0·89
Controls ($n = 4$)				
Mean ± SD	4·77 ± 1·50	1·08 ± 0·26	0·28 ± 0·09	1·85 ± 0·46

T, topical, S, systemic.

* Fibroblasts were labelled with [^{14}C]-proline for 24 h, after which hydroxyproline and total radioactivity were assayed as described, and are expressed per μg DNA.

† The ratio type III/type I collagen was determined from ^{14}C-proline-labelled medium collagen, after pepsin treatment and 8% SDS-gel electrophoresis using delayed reduction. The radioactive bands were quantified by densitometer.

‡ Fibroblasts at early confluence were harvested and used for the assay of prolyl hydroxylase as described.

§ $P < 0{\cdot}04$.

¶ $P < 0{\cdot}06$.

and systemically PUVA-treated cells and in the control cells. Because it is possible that UV irradiation may affect the relative synthesis of distinct collagen types,[24] the ratio of type III/type I collagen in the cell culture medium was measured after [^{14}C]-proline labelling, pepsin digestion and SDS-gel electrophoresis. The ratio of type III/I collagen in the fibroblasts from those topically and systemically PUVA-treated did not differ markedly from the corresponding controls (Table 2). The ratio of α1 (I): α2 (I) was also assayed in the same experiments, and found to be 2·08 ± 0·20 in topically treated cells, 2·0 ± 0·3 in systemically treated ones and 2·0 ± 0·3 in the controls, indicating that PUVA treatment does not affect the relative synthesis of the distinct chains of type I collagen or increase the synthesis of α1 (I) trimer.

As previous studies have indicated that UV irradiation may reduce the activity of prolyl hydroxylase, a key enzyme needed for the intracellular biosynthesis of collagen, this activity was also measured. It proved to be about the same in the topically and systemically treated cells and in the control fibroblasts (Table 2).

Measurement of mRNA levels of collagen, elastin and γ-actin

In order to study the relative collagen and elastin mRNA levels, total RNA was dotted on to nitrocellulose filters and hybridized with cDNA clones complementary to human pro α1 (I) collagen and elastin RNA sequences. Gamma-actin mRNA was measured as a reference RNA using cDNA for human γ-actin.

As the mRNA measurements were done in two separate experiments using different conditions, the comparisons can only be made within each experiment. The levels of mRNA of

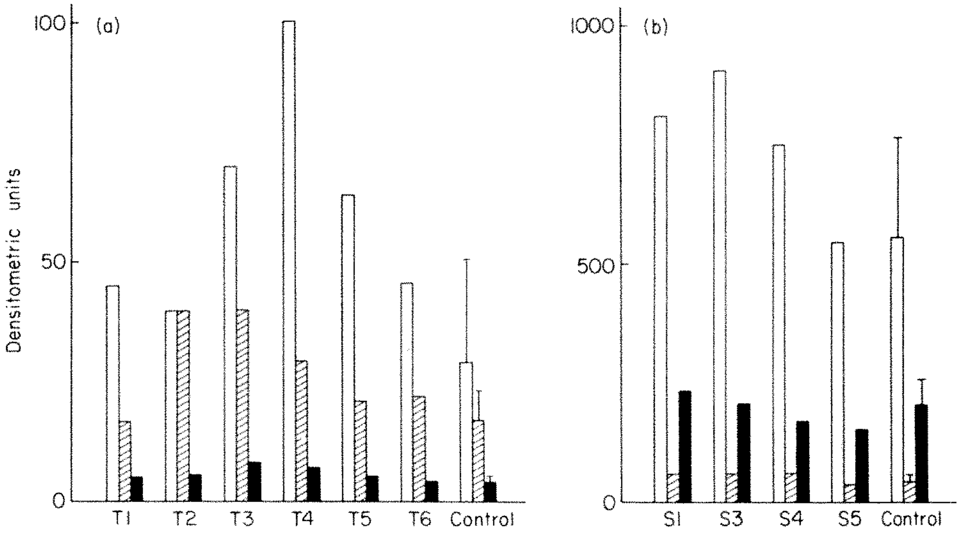

FIGURE 3. Collagen, elastin and γ-actin mRNA levels in fibroblasts derived from PUVA-treated and control subjects.

Total RNA was isolated and dotted on nitrocellulose filters and RNA was hybridized with ^{32}P-labelled pro α1(I) collagen, elastin and γ-actin cDNAs as described. The radioactive bands were quantified by densitometer.

The mRNA levels were measured in two separate experiments. In (a) the cells were derived from patients treated with topical PUVA and in (b) from patients treated with systemic-PUVA. The numbers under the figure refer to the cell lines described in Table 1. In each experiment the mRNAs were also measured from corresponding control fibroblasts; in (a) and (b) the number of controls was four. □, collagen mRNA; ▨, elastin mRNA; ■, γ-actin mRNA.

pro α1 (I) collagen and elastin were increased in PUVA fibroblasts when compared to corresponding control cells (Fig 3, Table 3). When collagen and elastin mRNA were expressed relative to the reference mRNA, γ-actin, there was a slight increase in the relative level of collagen mRNA in both topical PUVA and systemic PUVA fibroblasts, and in elastin mRNA in systemic PUVA fibroblasts, when compared with corresponding control cells (Table 3).

Collagenase activity

To study the degradation of collagen, collagenase activity was measured from the fibroblast media after brief trypsin activation of the latent collagenase. There was a slight increase in collagenase activity in the topically treated fibroblasts ($P < 0{\cdot}375$) (Fig. 4).

TABLE 3. The levels of collagen and elastin mRNA in control cells and cells derived from PUVA-treated subjects. The collagen, elastin and γ-actin mRNAs were measured as described in Methods and in the legend to Figure 3. The values between experiments 1 and 2 are not comparable, since the conditions and specific activities of probes were different

	Collagen mRNA (densitometric units)	*P*-value	Collagen mRNA/ actin mRNA	*P*-value	Elastin mRNA (densitometric units)	*P*-value	Elastin mRNA/ actin mRNA	*P*-value
Experiment 1								
Control ($n=4$)	28·5 ± 21·8	—	7·2 ± 3·9	—	17·0 ± 6·0	—	4·8 ± 1·6	—
T-PUVA ($n=6$)	61·2 ± 23·2	0·067	10·4 ± 2·8	NS	28·2 ± 9·9	NS	4·8 ± 1·3	NS
Experiment 2								
Control ($n=4$)	557·3 ± 214·1	—	2·6 ± 0·7	—	43·0 ± 9·8	—	0·2 ± 0·1	—
S-PUVA ($n=4$)	752·0 ± 151·8	NS	3·8 ± 0·5	0·029	54·0 ± 17·4	NS	0·3 ± 0·1	NS

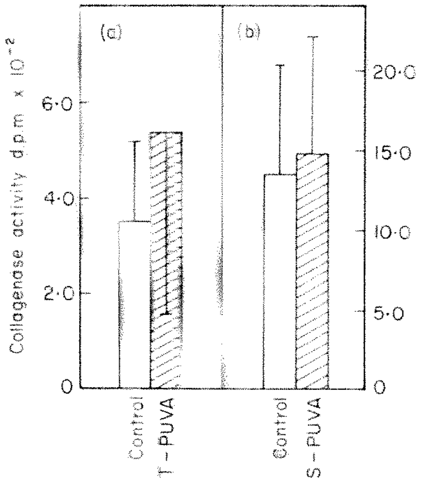

FIGURE 4. Collagenase activity in fibroblasts derived from PUVA-treated and control subjects. Medium fractions were used for the assay of collagenase activity after brief trypsin activation using type I collagen as the substrate. The results are expressed as degradation of [^{3}H]-proline-labelled type I collagen, DPM × 10^{-2}/μg DNA (mean ± SD). Number of cell lines: (a) control, $n=4$; T-PUVA, $n=6$. (b) control, $n=4$; S-PUVA, $n=3$.

Correlation of parameters measured to cumulative UV-dose

As there could be a correlation between changes in growth rate, synthesis of macromolecules and cumulative UV-dose, all the parameters measured (thymidine uptake, proliferation, attachment, collagen and total protein synthesis, collagen, elastin and γ-actin mRNAs, collagenase activity) were compared to cumulative UV-dose. There was no correlation between any of these parameters and the UV-dose in either topical or systemic PUVA-treated patients.

DISCUSSION

The proliferation rate of the fibroblasts derived from the skin of patients who had received systemic PUVA treatment was found to be decreased. This is in agreement with previous studies in which the life-span of fibroblasts was found to be decreased.[1] Psoralen photochemotherapy may affect the growth of fibroblasts *in vivo*, since UVA radiation penetrates into the dermis.[25] UV irradiation has also been shown to disrupt the cytoskeleton, that is important in the control of normal cell growth.[26]

The measurement of collagen and elastin mRNA levels by hybridization techniques has revealed that these mRNAs were increased in PUVA-treated cells. Histologically, UV-irradiation alone can cause an increase in elastin, and therefore it is possible that extensive and prolonged PUVA-treatment could cause an increase in elastin *in vivo*. The increase in collagen mRNA in cells derived from PUVA-treated patients is surprising. However, some studies have suggested that after UV-irradiation there are active fibroblasts that have a large endoplasmic reticulum.[27] Also, the alteration in collagen and elastin mRNA levels in fibroblasts obtained from PUVA-treated patients could be a secondary phenomenon due to decreased proliferation of the cells. For example, the level of collagen mRNA is dependent on the growth phase of fibroblasts[28] and therefore the altered growth rate as observed here could induce changes in collagen and elastin mRNA *in vitro*.

Incorporation studies using ^{14}C-proline have indicated that the relative synthesis of types I and III collagen was unchanged in the fibroblasts from those who had PUVA treatment. This indicates that PUVA therapy *in vivo* does not affect the gene expression of different collagen types of skin fibroblasts. This was further confirmed by the finding that the sizes of individual chains and the ratio of $\alpha 1$ (I)/$\alpha 2$ (I) was similar in the PUVA-treated and control fibroblasts. This provides indirect evidence that even long-term PUVA treatment does not induce marked changes in collagen gene expression, either by gene selection, mRNA processing, or translational efficiency of collagen mRNAs. This is supported by the observation that the activity of prolyl hydroxylase, a key enzyme in collagen synthesis, was unaltered in PUVA-treated skin.[29] The absence of any marked changes in the quantity of collagen[29] and elastin[1] as shown previously, indicates that collagen and elastin gene expresion *in vivo* is normal in these patients.

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Enhanced percutaneous absorption of a novel topical cyclosporin A formulation and assessment of its immunosuppressive activity

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SUMMARY

No clinically successful topical cyclosporin A (CyA) formulation has been produced, mainly due to the apparent lack of drug penetration. This study has produced the first *in vitro* kinetic data on CyA penetration across human cadavar stratum corneum and has shown that addition of the penetration enhancers (PE) Azone and propylene glycol to the CyA vehicle significantly enhanced drug permeation across the skin barrier. Using flow-through permeability cells with 5% w/v CyA (Sandimmun®) alone (CyA) or with PE (CyA + PE) in olive oil in the donor chamber, the penetration rate (mean ± SD $\mu g/cm^2/h$) into receptor fluid was 53 ± 43 ($n = 13$) for CyA and 660 ± 175 ($n = 7$) for CyA + PE.

The *in vivo* efficacy of this formulation was assessed in guinea-pigs undergoing delayed-type hypersensitivity (DTH) reactions to dinitrofluorobenzene (DNFB). CyA was applied topically at the time of challenge and twice daily thereafter. At 24 h, skin reactions revealed that compared with appropriate drug vehicles, concentrations of 0·25, 0·5 and 5% CyA ± PE had a significant inhibitory effect upon the erythema response and this corresponded with significant reductions in T-cell infiltrates (0·5 and 5% CyA). No statistically significant reductions in erythema were demonstrated with 0·05% CyA ± PE, but there was a reduction in the number of infiltrating lymphocytes in sites receiving 0·05% CyA + PE compared with vehicle-treated sites ($P < 0{\cdot}01$). This suggests that PE permitted some penetration of an otherwise non-immunosuppressive concentration of CyA through the skin.

Cyclosporin A (CyA), which is a fungal metabolite, is a potent inhibitor of T-lymphocyte-dependent cell-mediated immune processes. The systemic administration of this drug in low

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doses has been shown to be extremely beneficial in clearing chronic plaque psoriasis,[1,2] a disease of unknown aetiology, but which appears to have a T-cell involvement.[3] Despite its clinical success, dose-dependent, drug-induced hypertension and nephrotoxicity have been found in psoriatic patients on long-term CyA therapy for up to 3 years.[3,4] This has precluded use of the drug in less severe psoriasis and other immunodermatological disorders. These side-effects could be minimized or even abolished if CyA was administered topically. To date no effective formulation has been produced, primarily because little is known about the pharmacokinetics of percutaneous CyA absorption.

Although CyA has been applied topically to humans, at concentrations of 0·1–10% in oil or unguentum Merck vehicles, it was found to be largely ineffective in suppressing allergic contact sensitivity reactions,[5-7] in inducing hair regrowth in alopecia areata[8-10] or in reducing lesions in chronic plaque psoriasis.[11,12] The failure to produce a therapeutic response resulted from inadequate penetration of CyA through the skin, especially as no drug was detected in the blood.[7,10,12] Despite these failures, it has been shown that intralesional injection of CyA on 6 alternate days, produced a significant resolution of psoriatic plaques within 2 weeks.[13] This suggests that adequate amounts of locally applied CyA could be as effective as when the drug was administered systemically.

The principal barrier to percutaneous absorption is the stratum corneum, which functions as a rate-limiting factor in the penetration process, and which many drugs, including CyA, cannot cross.[14] Therefore solvents are usually incorporated into drug excipients to reversibly reduce the diffusional resistance of the stratum corneum. Water, alcohol, alkyl methyl sulphoxides, pyrrolidones, propylene glycol and Azone are all known penetration enhancers (PE) which collectively increase the hydration state and lipid fluidity of the stratum corneum.[15,16]

The purpose of this work was to formulate a topical CyA preparation incorporating suitable PE and to compare percutaneous absorption of CyA using this formulation with that of CyA without PE. An *in vitro* technique involving isolated stratum corneum placed in open flow-through permeability cells[17] was adopted. This method, which can be used to establish the steady-state passage of molecules, relies upon excess test solution being present on the donor (upper) side of the skin membrane, in order that the quantity of drug passing through the stratum corneum is negligible in proportion. Thereafter, the efficacy of the formulation was evaluated in guinea-pigs undergoing cutaneous delayed-type hypersensitivity (DTH) reactions. Unlike humans, where there has been little, or no, clinical response with topical CyA, guinea-pig skin appears to be relatively permeable to topically-applied CyA, since it effectively suppresses DTH responses[18] and reduces T-cell infiltrates.[19] The aim of this *in vivo* work was to ascertain whether the addition of PE, to an otherwise non-immunosuppressive concentration of CyA, could permit the penetration of sufficient drug through the skin to prevent elicitation of the DTH response.

METHODS

Drugs

CyA solutions were made from the 10% w/v oral preparation of CyA (Sandimmun, Sandoz Ltd, Basle, Switzerland) which was diluted with olive oil to the required concentrations of 0·05–5%. The CyA plus PE (CyA + PE) formulations contained in addition to CyA, 2% v/v Azone (AZ, Nelson Research, Irvine, CA, U.S.A.) and 18% v/v propylene glycol (PG, Sigma, Poole, Dorset, U.K.).

In vitro studies

Percutaneous absorption measurements were made using modified open flow-through permeability cells[20] containing stratum corneum prepared from human cadaver abdominal skin.[21] Upper donor chambers held 0·5 ml of 5% CyA + PE containing 30 μl ^{3}H-CyA (Amersham International PLC) of 370 kBq/ml activity. The total area of exposed stratum corneum in the cell was 0·2 cm^2. A non-ionic surfactant receptor solution of 6% w/v Volpo 20 in distilled water (Croda Chemicals Ltd., Goole, U.K.) was driven through the lower chamber (volume 80 μl) of the cell at 5 ml/h, thereby replacing the receptor fluid 63 times/h and maintaining sink conditions. The apparatus and solutions were maintained at 32 ± 1°C. The run-through receptor solution (2·5 ml) was collected every 30 min during a 5-hr period and the amount of ^{3}H-CyA penetrating the stratum corneum assessed by adding 10 ml PICO FLUOR 40 (Canberra Packard, Pangbourne, U.K.) scintillation fluid to these samples and counting them in a Packard Tri-Carb 4530 scintillation counter.

In vivo studies

Animals. Male Dunkin–Hartley guinea-pigs purchased from David Hall, Newchurch, Staffs, U.K. and weighing 700–900 g were used. They received a pelleted diet, supplemented with vegetables and hay.

Sensitization. Animals were sensitized to 1-fluoro-2,4-dinitrobenzene (DNFB) by epicutaneous application of 50 μl 10% (w/v) DNFB solution in acetone : olive oil (1 : 1) to the dorsum of the right ear.

Skin testing. Skin tests were performed 8 days after sensitization, by application of a non-irritant dose of 20 μl DNFB solution (0·5% w/v) in acetone : olive oil (4 : 1) to the shaved flank. The erythematous reactions were assessed 24 h later using the following scale: 4, red and elevated; 3, red but not elevated; 2, confluent pink; 1, patchy pink spots; 0·5, feeble reaction; 0, no change.

Topical regimen. Animals randomly received 20 μl topical applications of either 0·05, 0·25, 0·5 or 5% CyA ± PE, olive oil or olive oil + PE to three test sites on each flank, immediately after drying of the challenge DNFB (0 h). The drug was re-applied after 5 h and the animals were killed at 24 h when biopsies were taken for immunohistochemical analysis.

Immunohistochemistry. Following excision, a portion of each skin biopsy was embedded in OCT (Raymond A Lamb, London U.K.) and snap frozen in liquid Arcton, previously cooled to freezing point by immersion in liquid nitrogen. Cryostat sections (6–8 μm), cut in such an orientation as to include both the reaction 'epicentre' and outer margin of the reaction site, were mounted on alum–gelatin-coated slides and allowed to dry overnight at room temperature. Slides were stored wrapped in aluminium foil at −20°C then brought to room temperature before fixation for 20 min in acetone prior to staining.

The three-stage alkaline phosphatase–anti-alkaline phosphatase (APAAP) method was employed to demonstrate leucocyte phenotypes, using primary mouse monoclonal antibodies to guinea-pig mononuclear cells (Table 1). Secondary antibody, rabbit anti-mouse Igs (Dako, Copenhagen, Denmark) was diluted 1 : 20 in 10% normal guinea-pig serum.

TABLE 1. Monoclonal antibodies used in the immunocytochemical analysis of guinea-pig mononuclear cells

Antibody isotype	Specificity	Source	Dilution
CT7 IgG1	pan T cells	Dr R.J.Scheper*	1:2000
CT6 IgG1	T cytotoxic/suppressor cells	Dr R.J.Scheper	1:5000

* A gift from Dr R.J.Scheper, Pathological Institute, Free University Hospital, Amsterdam, The Netherlands.

Quantitative assessment of cellular infiltration. Cell counts in the epidermis and upper papillary dermis were performed blind on coded sections. Positive cells (in five consecutive areas encompassed by an eyepiece grid occupying *c.* 50% of the field of view) were counted using a × 10 eyepiece and × 25 objective.

Statistics

Results are expressed as means ± 1 standard deviation. The significance of differences between means was determined using Student's *t*-test (for cellular infiltrates) or Mann–Whitney non-parametric test (for erythema scores).

RESULTS

In-vitro penetration of CyA

The addition of Azone (AZ) and propylene glycol (PG) to 5% CyA enhanced the accumulation of ^{3}H-CyA in the receptor fluid during 5 h (Fig. 1a). The amount of CyA penetrating the stratum corneum in the absence of PE was 53 ± 43 μg/cm^2/h, whereas the addition of PE significantly ($P < 0{\cdot}001$) increased CyA absorption tenfold to 660 ± 175 μg CyA/cm^2/h. After 5 h, 2·6% CyA from the PE-containing formulation had penetrated the stratum corneum compared with only 0·2% from the CyA-only solution (Fig. 1b).

Delayed-type hypersensitivity reactions in the guinea-pig

Skin reactions. The influence of topical CyA, at different concentrations, either alone or in combination with PE, on the intensity of skin reactions to DNFB at 24 h is shown in Table 2. Examination of the guinea-pigs revealed that CyA concentrations between 0·25 and 5% were significantly immunosuppressive and that almost complete suppression of the DTH reaction occurred with 5% CyA. When CyA was applied at a concentration of 0·05% there was no inhibitory effect without PE, although a 30% reduction (which did not quite reach significance at $P < 0{\cdot}05$) in mean erythema score was achieved with PE compared with its vehicle. There were no significant differences in mean erythema scores between CyA and CyA + PE at each individual concentration of CyA. The PE themselves showed no immunosuppressive effect as the mean erythema scores for olive oil + PE were similar to sites receiving oil alone and when PE were applied at 20% v/v in saline to reaction sites in a separate experiment there was no reduction in erythema ($2{\cdot}3 \pm 0{\cdot}5$, $n = 6$) compared with saline-treated sites alone ($2{\cdot}4 \pm 0{\cdot}3$, $n = 6$).

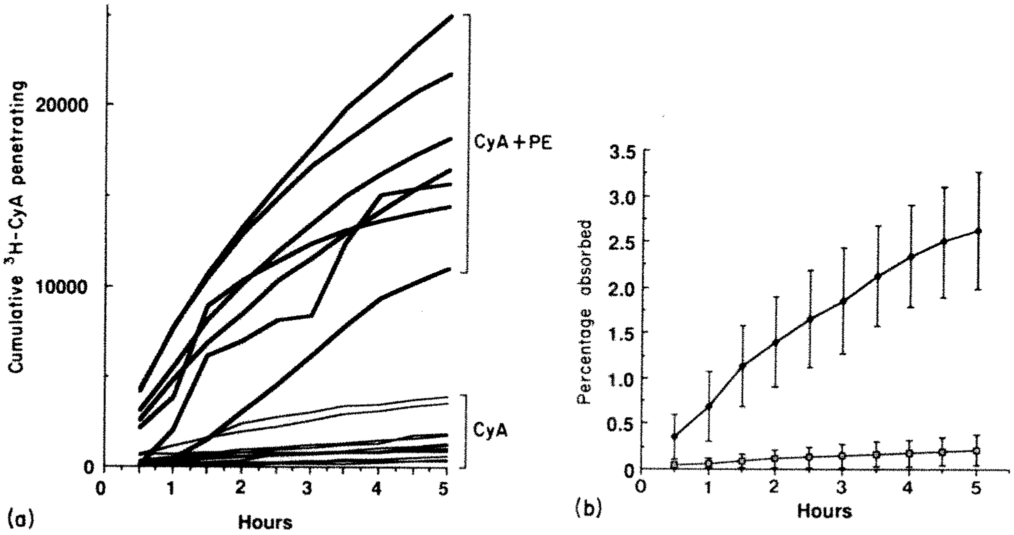

FIGURE 1. Penetration enhancers (PE = AZ + PG) enhanced the percutaneous absorption of CyA *in vitro* as shown by (a) cumulative ^{3}H-CyA counts for CyA alone ($n = 13$ wells) and CyA + PE ($n = 7$ wells); and (b) the mean ± SD percentage penetration of CyA alone (□) and CyA + PE (●) through stratum corneum during 5 h.

TABLE 2. Effect of topical CyA plus penetration enhancers on contact sensitivity reactions to DNFB

CyA concentration		Intensity of reaction	
(% w/v)	*n*	CyA	CyA + PE
Vehicle	10	2·8 ± 0·3	2·5 ± 0·4
0·05	5	2·6 ± 0·4	1·7 ± 0·6
0·25	4	1·9 ± 0·2**	1·8 ± 0·4**
0·5	3	2·0 ± 0*	1·3 ± 0·5*
5·0	5	0·8 ± 0·7**	0·6 ± 0·5**

Results are (*n*, number of test sites) mean erythema ± SD.

Reactions to DNFB assessed at 24 h.

Symbols represent statistically significant differences from vehicle-treated control sites (* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$ (Mann–Whitney *U*-test)).

Quantitative immunohistochemistry. The results from immunophenotypic analysis of the lymphocytic infiltrate in reaction sites from animals treated with CyA ± PE are shown in Figures 2 and 3. Direct comparisons between CyA and CyA + PE, at each concentration of CyA, for

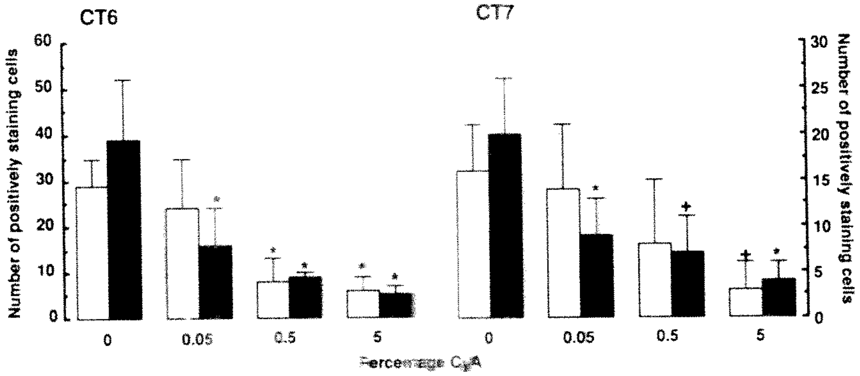

FIGURE 2. Effect of topical CyA ± PE on the numbers of CT6$^+$ and CT7$^+$ infiltrating lymphocytes at 24 h within DNFB-challenged skin sites in guinea-pigs. Results expressed as mean ± SD. Symbols represent statistically significant differences from vehicle-treated controls (Student's *t*-test). * $P < 0{\cdot}01$; † $P < 0{\cdot}02$. □, CyA; ■, CyA + PE.

CT7$^+$ (pan T cell) and CT6$^+$ (T suppressor/cytotoxic) lymphocytes revealed no significant differences in the mean number of infiltrating cells (Fig. 2). In contrast, when each concentration of CyA ± PE was compared with its own drug vehicle control, there were significant reductions in the numbers of CT6$^+$ and CT7$^+$ cells at 0·05, 0·5 and 5% CyA + PE but only at 0·5% (CT6 only) and 5% for CyA alone. Due to the greater mean number of lymphocytes in vehicle plus PE-treated sites compared with vehicle-treated alone (25% for CT7 and 35% for CT6), the reduction in infiltrating cells in skin sites receiving 0·05% CyA + PE assumed significance. These reductions over appropriate vehicle control values were 55% and 59% for 0·05% CyA + PE compared with only 12% and 17% for 0·05% CyA for CT7$^+$ and CT6$^+$ cells, respectively.

DISCUSSION

The results from this work demonstrate that Azone and propylene glycol (AZ + PG) significantly enhanced the penetration of CyA (560 μg/cm²/h; 2·6% after 5 h) through human stratum corneum. Chanez and Wepierre[22] have reported that the removal of stratum corneum by stripping resulted in more CyA penetrating the dermis (28%) than occurred with intact skin (1·5%) during *in vitro* penetration of a 10% solution of the drug. To date, no other *in vitro* kinetic studies comparable to our own and using isolated stratum corneum have been made with CyA. Two short reports have found that when 10% CyA was applied to full-thickness skin *in vitro*, by 48 h only 0·13% of the drug had penetrated through the skin itself in one study[22] while the other recovered 0·33% from the skin and receptor fluid.[23] Similarly, when 15% CyA was incorporated into an Azone-containing vehicle, Biren *et al.*[24] recovered a total of only 176 μg/cm² CyA after 48 h: percutaneous penetration (in receptor solution) 18 ng/cm², epidermal content 78 ng/cm² and dermal content 80 ng/cm². The detection of low amounts of CyA, by these authors, both in skin and receptor fluids demonstrates the problems of assessing percutaneous absorption with hydrophobic molecules when using full-thickness skin.

Normally, hydrophobic compounds that are absorbed through the skin are removed by blood in the capillaries immediately underlying the epidermis and usually do not penetrate the full

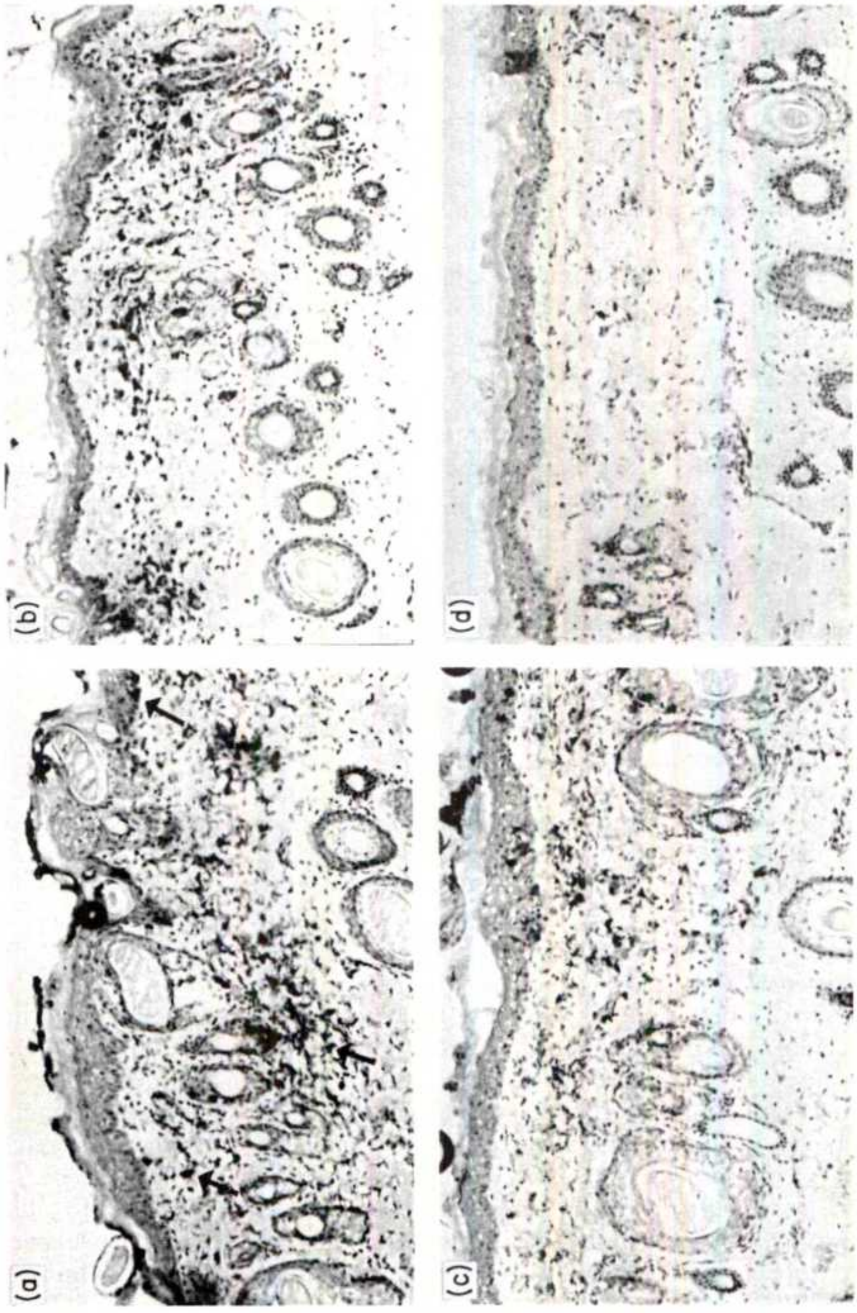

FIGURE 3. Immunohistochemical staining by APAAP of infiltrating CT6$^+$ staining cells at 24 h after DNFB-challenge from treated reaction sites receiving: (a) vehicle, (b) 0·05% CyA, (c) vehicle + PE and (d) 0·05% CyA + PE. Positively staining cells (arrowed) are conspicuous in the epidermis and upper dermis of (a), (b) and (c). (×80).

thickness of the skin. In addition the use of saline, which was described as a receptor fluid for CyA in one study,[23] may also be inappropriate, since lipophilic molecules are more likely to remain in the skin than partition into the aqueous receptor fluid. This latter point was highlighted by Bronaugh and Stewart[25] when they examined the effect of different receptor fluids on the permeation of hydrophobic compounds. They showed that considerably enhanced *in vitro* absorption through split-thickness skin was achieved, without affecting skin integrity, using a 6% solution of the non-ionic surfactant oleth 20 (Volpo 20) in water as a receptor fluid. As a result of these problems this study employed stratum corneum and used 6% Volpo 20 as a receptor solution.

The appropriate selection of PE is an important factor for maximizing percutaneous drug delivery. Using 10% CyA in an oily alcohol gel in one study, resulted in a marked improvement in 11 out of 18 patients with atopic dermatitis after 2 weeks' treatment.[26] Even when CyA was used at only 0·25% in an alcohol/propylene glycol-containing vehicle, complete hair regrowth resulted in a patient with alopecia areata following 16 months' treatment.[27] Azone by itself at 1–5% is a potent enhancer for both hydrophilic and hydrophobic drugs.[15] Wooton *et al.*[28] however, found that on addition of 18% PG as a cosolvent to 1% Azone there was an increase in transdermal absorption of metronidazole from 10 to 80% after 20 h. Based on our results, it would appear that a 2% AZ:18% PG solvent system is similarly effective in enhancing percutaneous absorption of CyA and would be safe for topical application in humans, as AZ has minimal irritancy when applied neat[29] and PG is widely used at similar concentrations in the pharmaceutical and cosmetic industry.

Our *in vivo* study showed that CyA, when applied topically at concentrations of 0·25–5·0% ± PE, inhibited contact-sensitivity reactions in the guinea-pig. This finding agrees with previous reports of topical CyA successfully attenuating DTH reactions in the guinea-pig when used at concentrations of 1–10% in olive oil[18,19,30] or at 15% in an Azone-containing vehicle.[31] This suppression is not due to any interference by the CyA with the contactant itself since the non-immunosuppressive dihydrocyclosporin A derivative, dHCyA-d, exhibited no inhibitory effect under similar experimental conditions.[19] Despite the profound immunosuppressive qualities of topical CyA in the guinea-pig, similar effects have not been reproduced clinically in contact sensitivity.[5,6] Extrapolating results for the efficacy of topical CyA formulations in the guinea-pig to humans therefore requires caution, especially since guinea-pig skin appears more permeable to CyA than human skin. It is for this reason we considered it important to titrate CyA to a concentration which no longer produced an immunosuppressive effect and then determine whether PE would enable more drug substance to penetrate the skin. Indeed, 0·05% CyA, which alone proved non-inhibitory, did on the addition of PE produce significant reductions in T lymphocytes during DTH reactions. This result emphasizes the point made by Anderson,[32] that macroscopic assessment of DTH responses does not necessarily correlate with the cellular infiltrate found within the reaction site. This was also demonstrated by the vehicle results which were similar macroscopically but microscopically sites receiving PE showed slightly greater cellular responses, possibly because the PE allowed more DNFB through the skin. Despite this, the incorporation of PE with 0·05% CyA appeared to sufficiently enhance drug delivery during the induction phase of the DTH reaction so that CyA was capable of suppressing lymphocytic infiltration by up to 90% compared with controls. Surprisingly, despite the observed reductions in lymphocyte numbers, no preferential depletion of either CT7$^+$ or CT6$^+$ cells resulted at any CyA concentration. Paradoxically, numerically more 'cytotoxic/suppressor' positive cells were present in sections than 'pan T' positive cells. This observation has been described previously[19] and discussed by Antoniou *et al.*,[33] who suggested that the antigen expression may be modulated on guinea-pig lymphocytes during immune responses.

In conclusion, the results demonstrate that this novel formulation of CyA displays enhanced penetration properties over CyA alone and in a laboratory-animal model is effective at low-drug concentrations in attenuating lymphocytic infiltration. Its possible clinical potential requires investigation in immunodermatological conditions, such as psoriasis.

ACKNOWLEDGMENTS

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Cyclosporin A in combination with photochemotherapy (PUVA) in the treatment of psoriasis

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SUMMARY

Forty patients with relapsing plaque psoriasis involving more than 20% body surface were treated either with cyclosporin A (CyA) plus PUVA or the retinoid etretinate plus PUVA (RePUVA). They initially received either CyA (2 weeks) or etretinate (1 week) alone and then PUVA was given concomitantly until complete remission. The patients were monitored over a period of 6 months and any relapse recorded. With each combined treatment regimen, CyA plus PUVA and RePUVA, the patients cleared within comparable periods of time (mean treatment period of 5·3 vs. 4·7 weeks after initiation of therapy and 3·3 vs. 3·7 weeks after initiation of PUVA). However, the cumulative UVA dose required for clearance (110·9 J/cm^2 vs. 62·1 J/cm^2 ($P < 0{\cdot}05$)) and the incidence of severe and early relapses were significantly higher in the CyA cohort. Within 6 months severe relapses had occurred in 58% of CyA plus PUVA but only in 15% of RePUVA-treated patients ($P < 0{\cdot}001$). This suggests that the CyA plus PUVA regimen as performed in this study is less effective than RePUVA.

The first report on the beneficial effect of cyclosporin A (CyA) in psoriasis appeared in 1979.[1] Since then the efficacy of CyA has been established in several open[2–8] and double-blind[9,10] trials. Unfortunately, short-term CyA treatment of psoriasis is followed by a high relapse rate[5,6] and it is apparent that long-term, low-dose CyA is required. However, extended CyA treatment may produce long-term side-effects such as nephrotoxicity,[2,11] and possibly an increased risk of lymphomas.[12,13] One approach to reducing the toxicity of a given therapeutic agent is to combine it with another drug with different side-effects. The rationale for such a combination is the augmentation of efficacy with a reduction of toxicity by non-additive adverse effects. Cancer polychemotherapy is based on this principle and in psoriasis a good example of such an approach

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is the combination of psoralen photochemotherapy (PUVA) with retinoids (RePUVA)[14,15] which has become standard therapy in a number of centres for severe psoriasis.

In the present study we investigated whether CyA can accelerate the response rate to PUVA in psoriasis, and whether PUVA reduces the relapse rate after stopping CyA. We have compared this combination of CyA and PUVA with an established RePUVA protocol using the response to PUVA as a criterion to evaluate the efficacy, and using the relapse rate to assess the stabilizing effect, of such a combination.

METHODS

Patients

Forty patients (29 male, 11 female; age range 24–74 years, mean 45 years) with severe plaque psoriasis involving more than 20% of the body surface were included in this study (Table 1). They were otherwise healthy with regard to their history and clinical and laboratory examination, except for two patients who had a history of a previous gastric ulcer. None of them had received any systemic antipsoriatic treatment or ultraviolet therapy for at least 4 months prior to the study, and none was taking any medication known to alter CyA metabolism. Female patients of childbearing age were excluded from the study. Informed consent was obtained and the patients were allocated to either CyA–PUVA or RePUVA treatment by non-random matching of age, skin type, severity and extent of disease.

Photochemotherapy (PUVA)

PUVA was performed according to an established regimen with 5-methoxypsoralen (5-MOP) (Geralen®, Gerot Pharmazeutika, Vienna, Austria) as described elsewhere.[16] Two hours after the ingestion of 5-MOP (1·2 mg/kg body weight) UVA irradiation treatment was given in a PUVA 6000 irradiation unit (H. Waldmann, Werk für Lichttechnik, GmbH, Schwenningen, F.R.G.) after determination of the minimal phototoxicity dose (MPD) in each patient. The patients were exposed four times per week, with rest days mid-week and at the weekend, until clearance of their psoriasis. The irradiation doses were adjusted according to the individual erythema reaction and the response rates of the psoriatic lesions. After clearance the patients received PUVA maintenance treatment consisting of twice-weekly PUVA irradiation for 4 weeks and once weekly for another 4 weeks. At this time maintenance PUVA therapy was stopped.

CyA plus PUVA

Twenty patients (Table 1) were treated with CyA (Sandimmun®, Sandoz, Basle, Switzerland)

TABLE 1. Demographic data

	CyA plus PUVA	RePUVA
Number of patients	20	20
Male/female ratio	13/7	16/4
Skin type (II/III ratio)	10/10	11/9
Age ($\bar{x} \pm s$)	45 ± 14	45 ± 13
Duration of psoriasis (years)	19 ± 16	20 ± 15

5 mg/kg body weight daily, in two divided doses. Blood levels of CyA (trough levels) were monitored twice a week by means of HPLC and ranged from 30 to 590 ng/ml (mean 153 ± 113 ng/ml, significantly lower than the therapeutic blood levels in kidney transplant patients of 200–400 ng/ml). During the first 2 weeks the patients received CyA alone. Thereafter, PUVA was initiated and given concomitantly with CyA until complete clearance was achieved. At this point CyA was discontinued and the patients were given PUVA maintainance therapy as outlined above.

RePUVA

Twenty patients (Table 1) were treated with etretinate (Tigason®, Tegison®, Hoffmann-La Roche Comp., Basle, Switzerland), 1 mg/kg body weight daily for 1 week according to our established RePUVA regimen.[17] Thereafter, PUVA therapy as outlined above was started and given concomitantly until complete clearance. Etretinate was then discontinued and PUVA maintenance therapy was given as outlined above.

Evaluation of treatment success and follow-up

Treatment success in both groups was assessed as a function of time of PUVA treatment, the number of PUVA exposures and the cumulative UVA dose delivered in the PUVA regimen until complete clearance was achieved. After clearance, the patients were monitored throughout the PUVA maintenance therapy period (2 months) and over an additional period of 4 months (i.e. 6 months after clearance) and relapses were recorded. During this period patients were allowed to use topical steroids for minor relapses. A recurrence of skin lesions covering more than 10% of the body surface was considered a severe relapse and patients were readmitted to PUVA or RePUVA treatment. Statistical analysis was performed using the Student's *t*-test.

RESULTS

CyA plus PUVA

Out of 20 patients, 17 patients were evaluated. Treatment of one patient was discontinued because of paresthesia, and in another because of lack of compliance. One patient did not respond to CyA and to the subsequent combination with PUVA therapy throughout a period of 6 weeks, despite therapeutic blood levels of CyA. In this patient CyA was discontinued and the psoriasis cleared after 4 weeks of treatment with RePUVA.

During the first 2 weeks of CyA treatment, the remaining 17 patients improved with respect to lesion thickness, scaling and erythema. After adding PUVA, complete clearance was achieved within a mean period of 3·3 weeks of PUVA treatment and a mean of 13·3 PUVA exposures. A mean cumulative UVA dose of 110·9 J/cm^2 was required to achieve this result. The mean time required for clearance from the initiation of CyA therapy was 5·3 weeks (Table 2). Six months after clearing four patients had remained in complete remission, three showed a recurrence of a few lesions and 10 had experienced severe relapses (Table 2). Six of these 10 patients had already relapsed during PUVA maintenance.

RePUVA

All 20 patients were evaluated. One week after initiation of etretinate the lesions had improved with respect to their scalyness but were unchanged with respect to lesion thickness and erythema as previously reported.[18,19] After initiation of PUVA patients were cleared within a mean period of 3·7 weeks, requiring a mean of 14·6 PUVA exposures and a mean cumulative

TABLE 2. Evaluation of treatment success and follow-up in CyA-plus-PUVA- and RePUVA-treated patients

	CyA + PUVA	RePUVA
Number of patients	17	20
Clearing time (weeks)	5·3 ± 1·5*	4·7 ± 1·0*
Duration of PUVA treatment (weeks)	3·3 ± 1·5*	3·7 ± 1·0*
Number of exposures	13·3 ± 6·0*	14·6 ± 3·7*
Cumulative UVA dose (J/cm²)	120·9 ± 101·5**	62·1 ± 22·6**
Initial UVA dose (J/cm²)	3·8 ± 1·7*	3·1 ± 1·0*
Mean single UVA dose	7·4 ± 4·1***	4·3 ± 1·2***
Patients with severe relapses within 6 months	10 (58%)****	3 (15%)****

*, Non-significant; **, $P = 0·05$; ***, $P = 0·005$; ****, $P = 0·001$.

UVA dose of 62·1 J/cm² (Table 2). The mean time required for clearance from initiation of retinoid medication was 4·7 weeks. After 6 months 16 patients had remained in complete remission, one had a recurrence with a few lesions and three patients had severe relapses (Table 2). None of these patients relapsed during the PUVA maintenance phase.

Side-effects

Side-effects occurred in only one of the CyA-treated patients. This was a 57-year-old man who developed tremor and paresthesia after 2 weeks of CyA treatment. CyA was stopped and the patient was excluded from the study. In the etretinate-treated patients the usual side-effects[18,19] occurred: dry skin, cheilitis and occasional dry rhinitis. These side-effects were well tolerated by the patients and subsided when retinoid treatment was stopped.

DISCUSSION

The precise mechanism of immunosuppression by CyA is still unknown. *In vitro* data suggest that CyA interferes with lymphokine-mediated (interleukin 2) T-lymphocyte activation,[20] and with antigen presentation by Langerhans cells[21] and macrophages.[22] It is also capable of modifying HLA-DR expression by endothelial cells[23] and of inhibiting proliferation of keratinocytes,[24,25] all of which are believed to be involved in one way or another in the pathogenesis of psoriasis. The exact mechanism of PUVA action is also unclear, but it is assumed to be related to an inhibition of DNA synthesis as a consequence of photoinduced DNA crosslinks.[26] PUVA-induced immunosuppression is thought to be related to a reduction in the number of antigen-presenting Langerhans cells[27,28] and the overall epidermal cell allostimulatory capacity.[28] PUVA may also decrease the number of circulating T cells, and may alter their function, perhaps as a result of impaired interleukin 2 production.[29,30] Thus the effects of CyA and PUVA may overlap, even though they may result from different mechanisms, and the combination of these therapies for psoriasis could result in a synergistic beneficial effect.

PUVA, etretinate and CyA are all effective in clearing severe plaque-type psoriasis when given alone but they differ in the speed by which this result is obtained. Clearance is most rapidly obtained by PUVA,[31] whereas the response to etretinate monotherapy is extremely slow.[18,19] The response to CyA is somewhere between PUVA and etretinate.[2,7,8,10] Since the combination of etretinate and PUVA (RePUVA) dramatically accelerates the response of psoriasis to PUVA,[14,15,17] we expected to see a similar acceleration when combining CyA with PUVA. Indeed, our results appear to demonstrate that CyA plus PUVA reduces the duration of PUVA treatment and the number of PUVA exposures required for clearance. This is clear when the results of the present study (3·3 weeks and 13·3 exposures required for clearance) are compared with those of another group of patients selected by identical criteria and treated at the same institution with an identical PUVA-protocol, but without any other concomitant therapy (5·3 weeks and 20 exposures required for clearance).[16] On the other hand, PUVA reduces the duration of CyA treatment required for clearing as is evident when our results (5·3 weeks) are compared to a patient group treated with an identical dose of CyA (5 mg/kg body weight) alone (9·2 weeks).[8] Thus, the combination of CyA and PUVA accelerates complete clearance, and is similar to the effect of retinoid–PUVA. The comparison of CyA plus PUVA and etretinate plus PUVA performed in this study shows that both treatment protocols were equally effective with respect to the time needed for clearing (5·3 vs. 4·7 weeks), the number of PUVA exposures (13 vs. 14 exposures) and the duration of PUVA treatment (3·3 vs. 3·7 weeks). However, in contrast to the 'Joule sparing' effect of the combination of etretinate plus PUVA,[14,15] the combination of CyA plus PUVA was associated with a significantly higher cumulative UVA dose (110·9 J/cm^2) as compared to the RePUVA cohort (62·1 J/cm^2). This cannot be interpreted as an effect of patient selection, because both groups were comparable with regard to skin type, age, history of psoriasis and severity of the disease.

The cumulative UVA load is a function of the patients' individual sensitivity to the phototoxic PUVA reaction and the response rate of the disease treated. Phototoxicity testing was performed in all patients prior to CyA and etretinate treatment. As both groups showed comparable MPD values, the initial UVA treatment doses were also comparable. The higher cumulative UVA radiation load in the CyA-treated patients was the result of markedly higher dose increments in this group. As dose increments are usually performed in order to maintain a faint erythema reaction,[31] we conclude that PUVA erythema was suppressed in the CyA–PUVA group. Studies are underway to test whether CyA indeed suppresses the UV irradiation-induced erythema reaction. Because previous observations have indicated that the efficacy of PUVA is maximized when the UVA dose is delivered in erythema threshold doses[31] it may well be that in this respect CyA antagonizes the PUVA effect. As the long-term side-effects of PUVA are related to the total cumulative UVA irradiation load, this CyA effect may increase the risk of UVA dose-related long-term side-effects of PUVA.

The second important issue in this study concerns the duration of remissions. There was a remarkably high incidence of severe and early relapses in the CyA group which was statistically significantly higher than in the RePUVA group. Moreover, severe relapses occurred in six CyA plus PUVA patients during PUVA maintenance treatment which is uncommon in those on RePUVA.[32] At present, we have no explanation for this phenomenon but it seems that PUVA does not suppress the tendency for early relapses in patients with psoriasis treated with CyA.[5,6]

In summary, CyA and PUVA are synergistic in the speed of clearing psoriasis but this is not dramatic enough to suggest that a CyA/PUVA combination represents a major step forward in the treatment of psoriasis. It is certainly not as effective as the results seen with RePUVA combinations because of the higher UVA doses required for clearance and the higher and earlier relapse rates. Taking into account that PUVA-induced skin tumours may be the result of a disturbed immune surveillance[26] and that the combination of PUVA with a potent immunosuppressant such as CyA may potentiate long-term risks of PUVA, we conclude that CyA–PUVA combinations cannot be recommended in the treatment of psoriasis.

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A case of T-cell lymphoma associated with panniculitis, progressive pancytopenia and hyperbilirubinaemia

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SUMMARY

A 57-year-old woman with T-cell lymphoma developed subcutaneous tumours and panniculitis and also a progressive pancytopenia.

Subcutaneous tumours and panniculitis have been reported in association with T-cell lymphoma. Rarely, Weber–Christian disease in which a panniculitis occurs can be associated with a progressive pancytopenia and haemophagocytosis.[1–5] We report a patient with T-cell lymphoma that was associated with panniculitis with a progressive pancytopenia caused by haemophagocytosis in the bone marrow.

CASE REPORT

In 1988, a 57-year-old woman presented to the Tottori University Hospital with a 3-year history of multiple subcutaneous tumours that were non-tender and were present on her face, neck, back, legs and arms. These varied in colour from normal to dark red. Her upper lip was indurated and oedematous. Some of the nodules disappeared spontaneously to leave areas that were slightly hyperpigmented. Treatment with prednisolone produced no improvement. She was apyrexial and the oropharynx and vocal cords were oedematous. The lymph nodes in the axillae were palpable.

Laboratory investigations revealed a haemoglobin of 11·0 g/dl, red cell count of 362×10^4/mm^3, white cell count of 3100/mm^3, lactic dehydrogenase 277 U/l and an anti-DNA antibody titre of 1/320. A gallium isotope scan showed that the subcutaneous nodules were hot spots. Ultrasound indicated hepatosplenomegaly and a fatty liver but the fatty liver was not confirmed on computerized tomography. An enlarged heart with a pericardial effusion was also shown on

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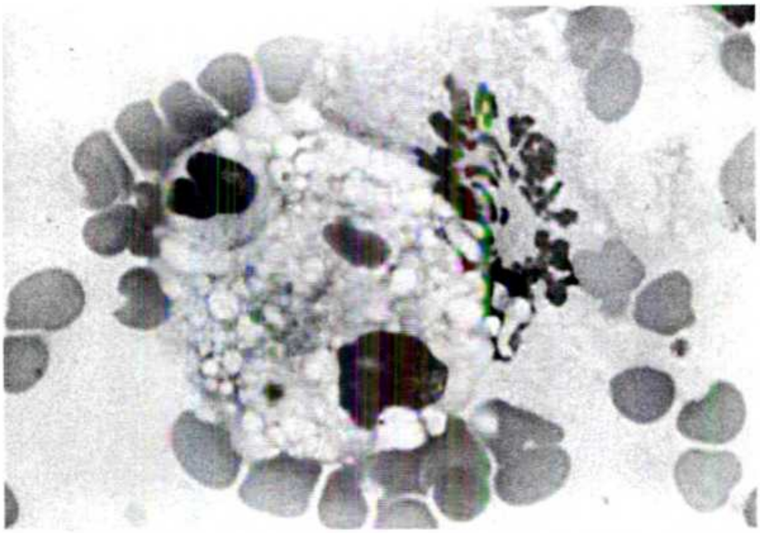

FIGURE 1. Haemophagocytosis in the bone marrow with a phagocyte surrounded by erythrocytes (× 1000).

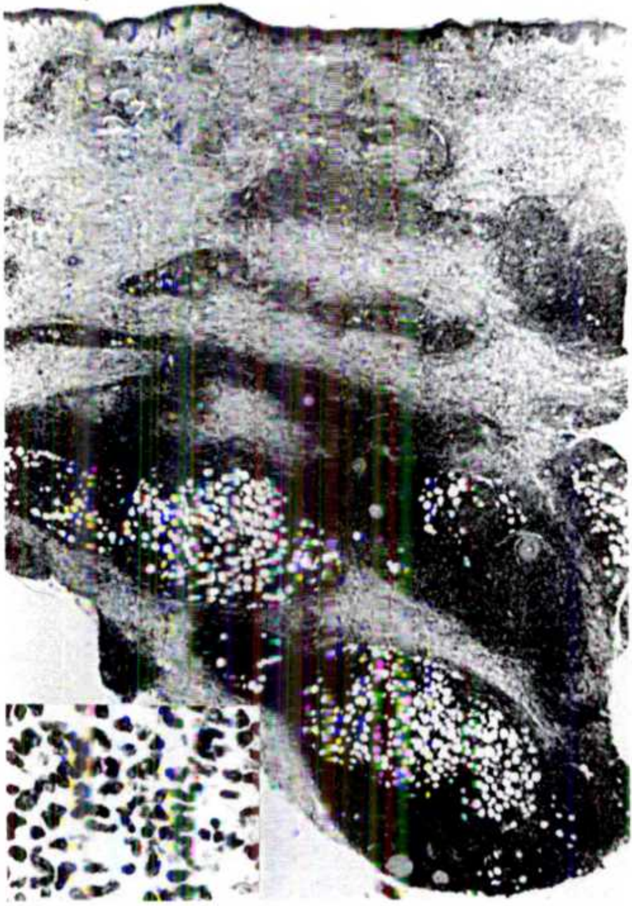

FIGURE 2. Atypical lymphocytes invading deep into the dermis and involving subcutaneous fat (× 20). Inset: the infiltrating cells show cleaved and hyperchromatic nuclei (× 330).

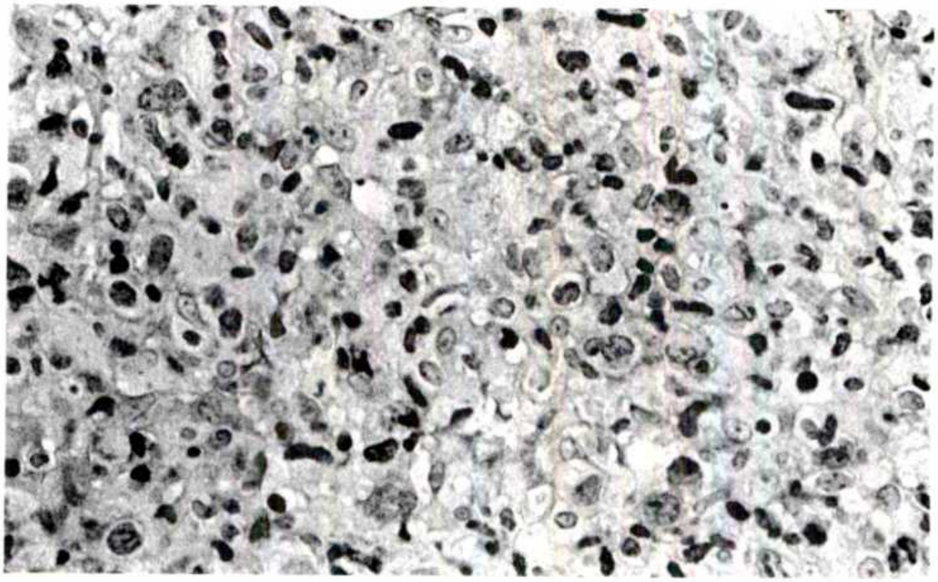

FIGURE 3. Monoclonal antibody UCHL-1 (T-cell marker) labelling of the infiltrating cells in subcutaneous fat (× 510).

ultrasound. Lymphangiography indicated that the lymph nodes were not involved. A bone marrow biopsy taken from the iliac crest revealed atypical lymphocytic infiltration with haemophagocytosis (Fig. 1).

A skin biopsy from a subcutaneous nodule on the left arm showed a panniculitis with a diffuse infiltrate of atypical lymphocytes (Fig. 2) that were also invading into the epidermis, small vessels, skin appendages and nerves. These lymphoid cells had indented nuclei and immunohistochemical studies showed cells that were labelled by the following monoclonal antibodies: CD2 (100% positive cells), CD4 (80% positive cells), B1 and B2 (0%). Cells infiltrating into the subcutaneous fat and labelled with the monoclonal antibody UCHL-1 are shown in Figure 3. On electron microscopy the cells had irregular shaped nuclei with abundant heterochromatin that was condensed towards the nuclear membrane (Fig. 4).

Following her admission to hospital, the pancytopenia and hypoproteinaemia rapidly progressed and the white cell count fell to 900/mm^3 and the platelet count to $2{\cdot}7 \times 10^4$/mm^3. The serum protein level fell from 5·3 g/dl to 3·9 g/dl. She was treated with vincristine, endoxane, prednisolone and adriamycin and the multiple subcutaneous nodules reduced in size. However, 4 weeks later there was rapid enlargement of the lymph nodes, liver and spleen with a rise in the level of the serum lactic dehydrogenase and bilirubin. A second course of treatment with novantrone, cyclophosphamide, vincristine and prednisolone reduced the lymphadenopathy and serum lactic dehydrogenase level, but the hyperbilirubinaemia did not respond. The endophagocytosis of the bone marrow increased and the patient died. No post mortem examination was performed.

DISCUSSION

Panniculitis may be associated with Weber–Christian disease, Rothman–Makai syndrome, malignant lymphoma, infections and immunological disorders.[1-6] In our patient with T-cell

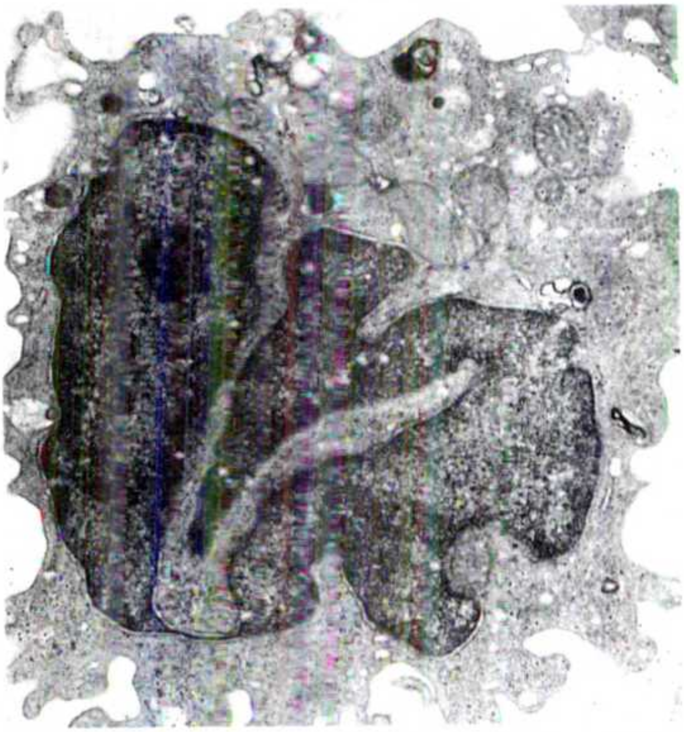

FIGURE 4. Electron micrograph showing a tumour cell with an irregular cleaved nucleus with abundant heterochromatin that is condensed towards the nuclear membrane (× 13,000).

lymphoma atypical lymphocytes were found to have invaded the subcutaneous fat and she had a panniculitis. Erythrophagocytosis has been reported in malignant histiocytosis, Weber–Christian disease and malignant lymphoma,[5,7] and these disorders when accompanied by cytophagia are often rapidly fatal.[3] However, several cases have been reported of cytophagic histiocytic panniculitis that have not had a fatal outcome.[8] In our patient, the characteristics of the haemophagocytes in the bone marrow could not be determined, but it is of interest that their number increased and that erythrophagocytosis was accelerated following chemotherapy.

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Treatment of severe acne with isotretinoin in patients with inflammatory bowel disease

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SUMMARY

Four patients with inflammatory bowel disease and severe cystic acne were treated with isotretinoin. Two patients had a successful course of treatment without any gastrointestinal side-effects. One patient had two episodes of profuse rectal bleeding that were probably related to pre-existing haemorrhoids. The fourth patient had a flare-up of his Crohn's disease after starting isotretinoin.

Patients with severe acne and chronic inflammatory bowel disease present a therapeutic dilemma. Although isotretinoin is an accepted treatment for severe acne, it is reputed sometimes to cause inflammatory bowel disease, although experienced physicians have not observed this association.[1] Oral antibiotic therapy for acne may aggravate chronic inflammatory bowel disease and systemic steroids that are often necessary for the treatment of this disorder may exacerbate acne. Although in our experience patients with severe acne and chronic inflammatory bowel disease are infrequently seen, we report four patients with this association in whom we considered that isotretinoin was the treatment of choice.

CASE REPORTS

Case 1

A 17-year-old man with a 1-year history of Crohn's colitis had severe cystic acne that was not responsive to oral antibiotics and was on prednisolone 10 mg daily and predsol enemas for moderately active bowel disease. After a 6-month course of isotretinoin at a dose of 0·7 mg/kg body weight daily his acne completely cleared. There was no adverse effect on his Crohn's disease.

Case 2

A 16-year-old man had a 6-year history of Crohn's disease that affected the terminal ileum

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which was currently in remission while on prednisolone 5 mg on alternate days, and he had severe inflammatory acne that had been unresponsive to oral antibiotics. This cleared completely with a 4-month course of isotretinoin (0·77 mg/kg body weight). This patient had no gastrointestinal side-effects while on the drug.

Case 3

A 31-year-old male had a 5-year history of ulcerative colitis that affected the left side of the colon and which was mildly active while on prednisolone 7·5 mg daily. He was also having predfoam enemata and was on sulphasalazine and metronidazole. He had intermittent rectal bleeding from haemorrhoids and a long history of inflammatory and scarring acne that had been unresponsive to oral and topical antibiotics. His acne improved with a 4-month course of isotretinoin at a dose of 0·85 mg/kg body weight, but subsequently became active again and required a further 3-month course of therapy. Although there was no worsening of his colitis, the treatment was terminated when he had profuse rectal bleeding on two occasions which on investigation was almost certainly from haemorrhoids.

Case 4

A 20-year-old male with a 2-year history of Crohn's disease affecting the distal colon had severe cystic acne. Oral antibiotics had caused an exacerbation of his Crohn's disease and he was started on isotretinoin, receiving 0·77 mg/kg body weight. On three occasions his colitis became worse within 2–6 weeks of starting isotretinoin. This necessitated stopping treatment. Each exacerbation lasted 4 weeks and settled without specific therapy.

Rectal biopsy histology

All three patients with chronic inflammatory bowel disease that involved the colon had mild to moderate goblet cell depletion with an excess of chronic inflammatory cells in the lamina propria in the pretreatment rectal biopsies. Repeat biopsies performed just prior to cessation of treatment with isotretinoin showed no change in goblet cell morphology or number and the degree of inflammatory cell infiltrate. Granulomata were noted for the first time in the late treatment biopsy in Case 4. Both the pre- and the late-treatment biopsies in Case 2 that had Crohn's ileitis were normal.

DISCUSSION

There are a few reports in the literature of patients with chronic inflammatory bowel disease and acne who have been treated with isotretinoin and suffered no adverse effects on the bowel disease.[2–4] Three of our patients experienced no gastrointestinal disturbance during isotretinoin therapy. The fourth patient who had mildly active disease prior to therapy had an exacerbation of bowel symptoms that appeared to have a temporal relationship to his acne treatment. Thus, although isotretinoin may not alter the clinical course of chronic inflammatory bowel disease in most patients, a few might be intolerant to the drug.

The histological changes within the sebaceous glands caused by isotretinoin are well established.[5,6] We considered the possibility that if isotretinoin was to exacerbate chronic inflammatory bowel disease it might do this by 'drying' the bowel mucosa, possibly by causing atrophy of the goblet cells. Pretreatment rectal biopsies showed the expected decrease in the number of goblet cells in three patients with chronic disease, but no obvious further decrease in the late-treatment biopsies. Further confirmation of the lack of effect of isotretinoin on the rectal mucosa is provided by the absence of change in goblet cell morphology and number in the normal pre- and late-treatment biopsies in Case 2. This study does not, however, rule out the possibility of an altered physiological function of the goblet cells.

We conclude that isotretinoin can be safely given to patients with chronic inflammatory bowel disease. If any patient shows an exacerbation of their bowel disease that might be related to the drug therapy, isotretinoin should be discontinued.

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Multiple glomus tumours: successful treatment with electron beam irradiation

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SUMMARY

A case is reported of a 23-year-old male with multiple glomus tumours that showed marked involution after he had electron beam therapy.

Multiple glomus tumours of the skin are rare, the number of lesions usually being not more than 10. When the number is large, they are disseminated over the body and there is no particular grouping.[1] We report a male patient with numerous tumours that resembled racemose cavernous haemangiomata and which were widely distributed on the left side of the body. Following treatment with electron beam irradiation these lesions regressed.

CASE REPORT

A 23-year-old man presented with a number of bluish nodules on his left upper arm that appeared at the age of 12. Subsequently, similar lesions developed on the left forearm, increasing gradually in size and in number. Several bluish macules were noted on his left upper back at the age of 18. There was nothing relevant in his family and in his past history.

On examination, all the lesions were on the left side of the body. There were numerous dark-blue to slightly bluish, soft and compressible nodules that ranged from 5 mm to 2 cm in diameter. These were arranged in clusters on the back of the hand, the extensor surface and inner aspect of the limbs and on the upper back. In the left scapular region there were blue patches of skin with telangiectasia. The clustered tumours resembled racemose cavernous haemangiomata (Fig. 1a). There was no history of spontaneous pain in these lesions, although they were tender in some areas to palpation. The results of routine laboratory investigations were all within the normal range.

Histology

A biopsy taken from the left forearm showed on histology many irregular vascular spaces lined

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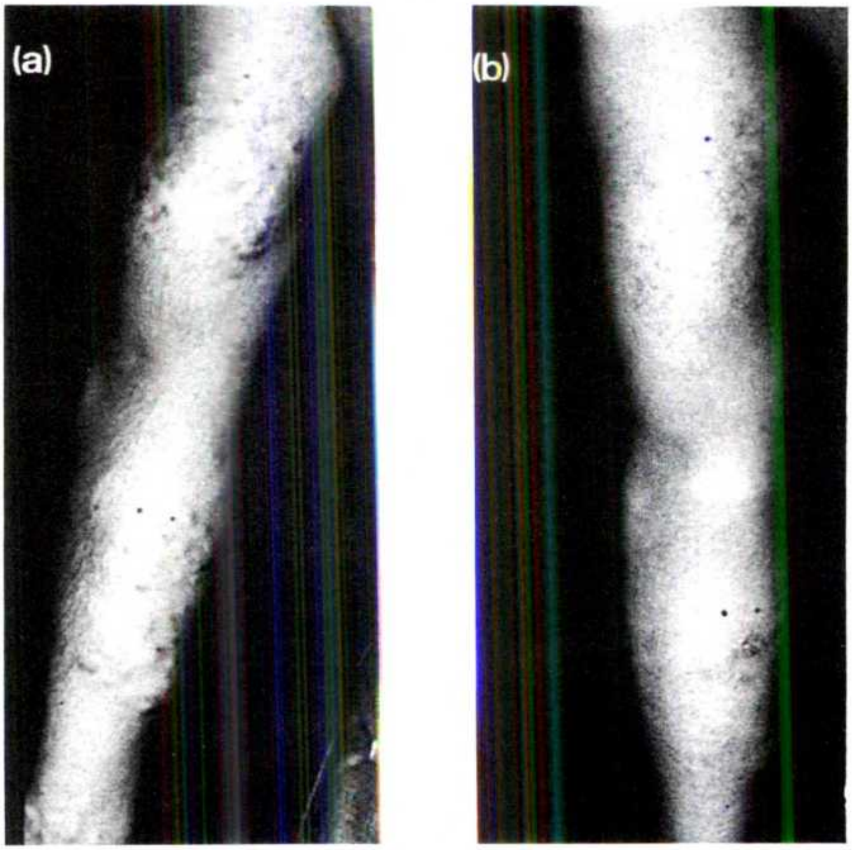

FIGURE 1. (a) Many bluish nodules aggregated on the left arm. (b) Almost complete flattening of the tumours 2 years after electron irradiation.

with flattened endothelial cells in the lower part of the dermis. Around the endothelium were strands and nests of cuboidal cells with relatively small and rounded nuclei that had the appearance of glomus cells (Fig. 2). Another skin biopsy specimen from the left upper back showed the same histology with a more prominent proliferation of glomus cells around the vascular lumen.

On electron microscopy, a single layer of endothelial cells was seen surrounding the vascular lumen and which was separated from the glomus cells by a thin layer of collagen. The tumour cells were round or polyhedral in shape with a round or oval nucleus which occasionally showed an indented margin (Fig. 3). The cells were enveloped by a relatively thick basal lamina. The cytoplasm of the cells contained a moderate number of mitochondria with abundant fine filaments that formed focal dense bodies. Numerous pinocytotic vesicles were seen, mainly along the plasma membrane of the glomus cells.

Progress and treatment

In October 1987 the patient was commenced on a course of radiotherapy and a total dose of 40 Gy was given over a period of 4 weeks to the lesions on the left forearm and upper arm at 10 MeV electrons. The lesions in the scapular area were treated over 3 weeks having 30 Gy at 8 MeV

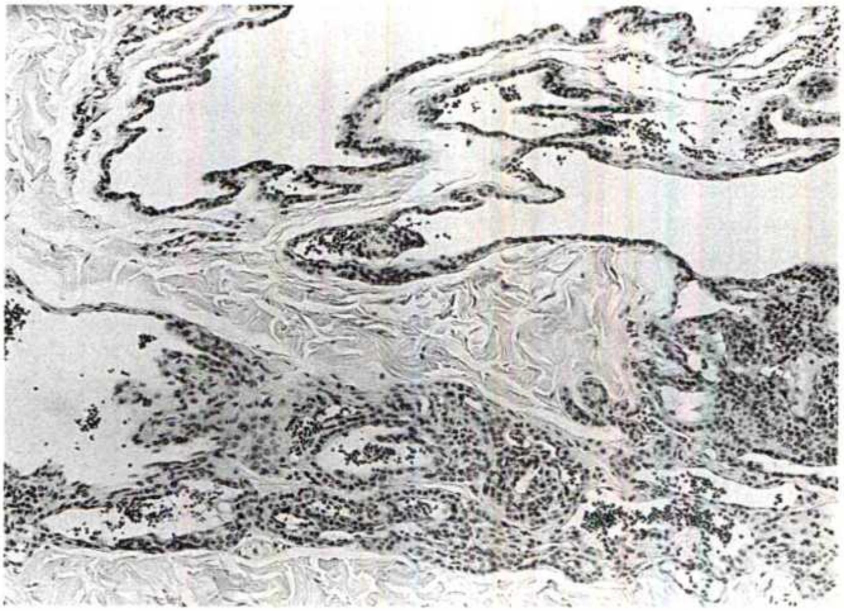

FIGURE 2. Glomus tumour showing vascular spaces lined with endothelial cells and with one to several layers of glomus cells around them (× 65).

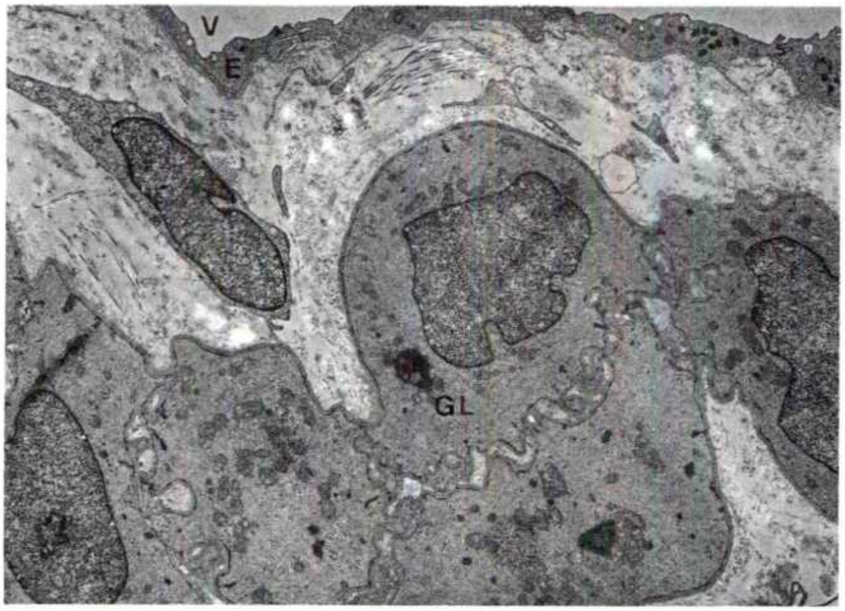

FIGURE 3. Glomus tumour cells (GL) surrounded by basement membrane. Endothelial cell (E) and vascular lumen (V) (× 4394).

electrons. There was only a slight decrease in the tumour bulk 3 months after the end of treatment, but at follow-up 17 months later the tumours had almost completely flattened (Fig. 1b).

DISCUSSION

Multiple glomus tumours are uncommon and are classified into two types which may be localized and generalized.[1] However, there are intermediate variants. In the localized type, lesions are located in a limited area such as on the hand or foot. They are usually painful and their histology resembles that of the solitary glomus tumour. In contrast, the generalized type may involve any area and the lesions are not usually tender and appear more angiomatous on histology.[1-3] Their number can vary considerably but rarely exceeds 10 in the localized type, and may also be low in cases of the generalized type. The tumours tend to be partially grouped together but can be very numerous, numbering over 400 in one report.[4] They are however discrete and can usually be counted. Our patient had numerous clustered tumours and so far only two similar cases have been reported in the Japanese literature.[5,6]

The histogenesis of multiple glomus tumours remains controversial and little ultrastructural difference can be found between the tumour cells in solitary glomus tumours and in multiple glomangiomas. In solitary glomus tumours, the basic structure of the normal glomus is more or less maintained, and these lesions are to be found where the cutaneous glomus occurs. Multiple glomus tumours however develop in sites where the normal glomus body is sparse or absent and there is usually a considerable vascular component. Multiple glomus tumours have been assumed to develop from tissue rudiments[7] and from the normal arterio-venous anastomoses.[8] There is a sparse innervation in multiple glomus tumours that is a minor difference from arterio-venous anastomoses.

The multiple lesions and the unilateral occurrence of the tumours as seen in our case and in others[2,9-11] suggests the result of a developmental defect in the embryo.[7] The onset of multiple glomus tumours in childhood[12,13] and its association with other malformations[2,4,14] suggests that they are of a hamartomatous nature.

It is stated that glomus tumours are insensitive to irradiation and should be treated surgically. However, a patient was described who had painful tumours that became painless following grenz ray therapy, although there was no reduction in their size.[10] Radiation therapy for glomus tumours involving the temporal bone (glomus jugulare) has been reported to prevent the further progression of signs and symptoms and the recommended dose was 45–50 Gy.[15] Although the gross angiographic appearance of the tumour did not change significantly, vessel damage with fibrosis was observed at autopsy after irradiation.[16] In our case, the cause of the involution of the tumours was not determined although it is likely that degenerative changes in the stroma of the tumour were involved.

Although malignant transformation of glomus tumours is extremely rare,[3] long-term follow-up of our patient will be necessary to confirm that there has been no regrowth or malignant change.

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Pachyonychia congenita complicated by hidradenitis suppurativa: a family study

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SUMMARY

A family is described in which five of the six members with the Jackson–Lawler type of pachyonychia congenita also had varying degrees of hidradenitis suppurativa. We suggest an association between this type of pachyonychia congenita and hidradenitis suppurativa.

Pachyonychia congenita is a rare genodermatosis which is characterized by a variety of ectodermal abnormalities. An autosomal pattern of inheritance is usual. Two major and a number of minor forms are recognized. The Jadassohn–Lewandowsky or type 1 variety consists of pachyonychia, palmar and plantar hyperkeratosis, hyperhidrosis, a propensity for the development of friction blisters, follicular hyperkeratoses and oral leucokeratoses. Distinctive features of the Jackson–Lawler, or type 2, variety are the presence of natal teeth and multiple cysts on the trunk, axillae, neck and scalp which are thought to represent epidermoid cysts and steatocystoma multiplex. Oral leucokeratoses are not generally observed, but the other abnormalities as seen in the Jadassohn–Lewandowsky type may occur.

CASE REPORTS

Six members of one family were identified as having pachyonychia congenita (Fig. 1). Each affected member had thickened, discoloured nails (Fig. 2) and hyperkeratosis with fissuring of the feet from childhood, leading to pain and difficulty in walking. With the exception of a 12-year-old girl (Fig. 1, III.1) all had hyperhidrosis, blistering of palmar and plantar surfaces and multiple epidermoid cysts on the neck and scalp and multiple steatocystoma of back, arms, axillae and chest. Two family members (Fig. 1, III.2 and III.3) also had numerous periorbital milia and one member (Fig. 1, III.4) had two incisors present at birth.

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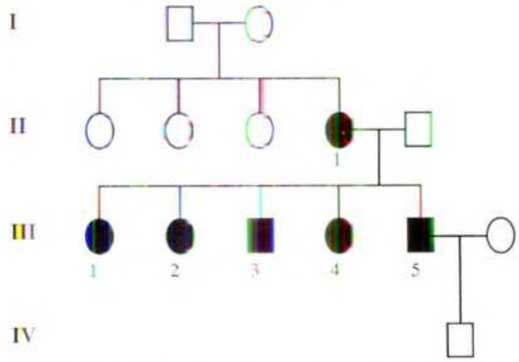

FIGURE 1. Pedigree of family. Circle, female; square, male; shaded symbols, evidence of pachyonychia congenita; open symbols, unaffected.

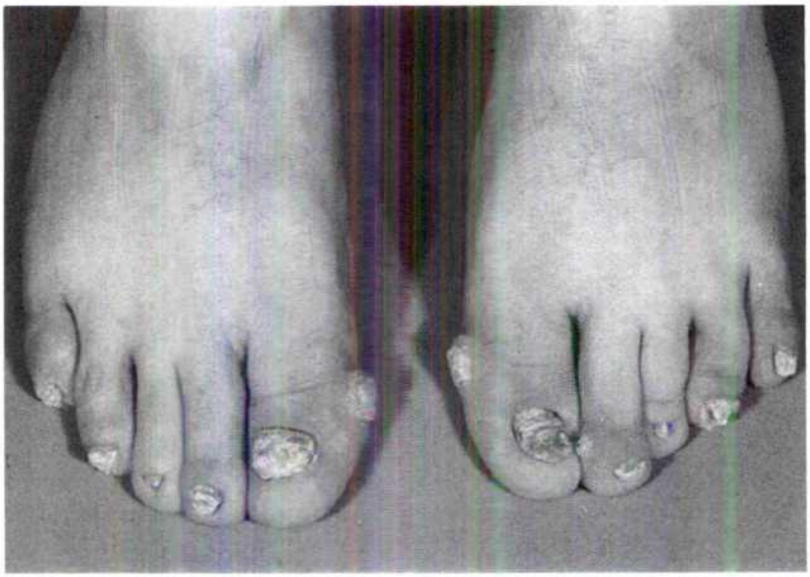

FIGURE 2. Typical thickened, dystrophic nails and hyperkeratosis (Case III-3).

Five of the six affected family members gave a history of recurrent abscesses particularly in the axillae, groins and anogenital areas (Table 1). Three patients had evidence of scarring, nodules and sinus track formation in the axillae. Although two male family members with pachyonychia congenita had a past history of mild facial acne, none of the females gave a history of androgen-related disorders.

Investigations

Biopsies of small cysts on the ear and arm were obtained from two members of the family. The histological appearance was consistent with the clinical diagnosis of epidermoid cysts. Patient III.4 (Fig. 1) developed an inflammatory lesion on the pinna which was excised and the

TABLE 1. Suppurative skin conditions and treatments of affected family members

Case	Sex	Age	Inflammatory skin conditions	Age at onset	Sites	Treatment	Examination
II-1	F	53	Recurrent abscesses	30	Axillae Anogenital	Antibiotics	No active suppuration
III-2	F	21	Recurrent abscesses	17	Axillae Groins	Antibiotics	No active suppuration
III-3	M	23	Recurrent abscesses	17	Axillae Groins	Antibiotics	Hypertrophic, tethered scars and hyperpigmentation
III-4	F	25	Recurrent abscesses Inflamed epidermoid cyst Discharging sinuses	19	Axillae Groins Scalp	Antibiotics; excision and grafting of axillary skin	No active suppuration
III-5	M	31	Recurrent abscesses Discharging sinuses	21	Axillae	Antibiotics, isotretinoin	Sinus tracks and scarring

histology was suggestive of an infected epidermoid cyst. One patient (Fig. 1, III.4) underwent excision and grafting of the axillary skin. Histological examination of the excised areas was consistent with the clinical diagnosis of hidradenitis suppurativa.

The serum levels of IgG, IgM and IgA were found to be within normal limits. The total neutrophil and lymphocyte counts were normal. No abnormality was detected in the numbers of T helper, T suppressor or natural killer lymphocytes or in the total number of B cells. A number of neutrophil functions were examined: random migration, chemotaxis, phagocytosis, and intracellular killing. No significant difference was noted between subjects and controls. Nail cultures were negative for fungi, yeasts and bacteria.

DISCUSSION

The family described here have the typical features of pachyonychia congenita of the Jackson–Lawler type. In addition, recurrent and chronic suppuration was a consistent feature in five of the six affected family members. The apocrine gland-bearing areas including axillae, anogenital regions and groins were particularly affected and varying degrees of scarring were observed.

An association between hidradenitis suppurativa and pachyonychia congenita has not previously been documented. Hidradenitis suppurativa is characterized by chronic suppuration and eventual scarring of apocrine gland-bearing skin. Apocrinitis associated with abscess formation and development of sinus tracks occurs. The primary event, however, is generally thought to be ductal occlusion by hyperkeratosis at the mouth of the apocrine follicle.[1,2]

Defects in systemic immunity are generally not thought to be important in the pathogenesis of hidradenitis suppurativa,[3] although it was recently noted that a small subgroup of patients with moderate and severe disease had reduced levels of T-helper cells.[4] We found no abnormality in neutrophil numbers and functions or in total T and B lymphocyte numbers. The subsets of T lymphocytes and immunoglobulin levels were also entirely normal. This suggests that the apparent predisposition of these family members to recurrent abscess formation and hidradenitis suppurativa was unlikely to be attributable to a shared immune defect.

There are four possible explanations for the apparent association between pachyonychia congenita and hidradenitis suppurativa. Inflammatory change occurring in steatocystomas located in the axillae and groins may lead to the development of hidradenitis suppurativa in these areas. This could account for the development of hidradenitis suppurativa in two patients described by McDonald and Reed,[5] who had natal teeth, epidermoid cysts and steatocystoma multiplex but no other features of pachyonychia congenita. An alternative explanation is that the follicular hyperkeratoses of pachyonychia congenita may predispose to the development of hidradenitis suppurativa by causing occlusion of the mouth of apocrine follicles. This seems improbable, as most patients with pachyonychia congenita or other hyperkeratotic conditions do not develop hidradenitis suppurativa. The coexistence of the two conditions in this family may of course be due to chance. However, since familial hidradenitis is recognized,[6] the fourth and most likely explanation is that a genetic linkage exists between the two conditions.

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Profound digital collagen atrophy: a new cutaneous presentation of adrenal-dependent Cushing's syndrome

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SUMMARY

A 59-year-old Caucasian housewife presented with a 2-year history of marked loss of tissue substance from the finger and toe pulps and the heel pads. There was no clinical evidence or history of urticaria or other inflammatory change. Investigations demonstrated a raised plasma cortisol secondary to a left adrenal adenoma. Skin biopsies showed abnormalities of dermal collagen, but no evidence of elastin destruction. This case presents an unusual variant of the cutaneous atrophy associated with Cushing's syndrome.

In his 1932 description of the clinical complex that now bears his name, Harvey Cushing[1] observed that its clinical manifestations were 'so many and varied as to baffle analysis'. Here we report a case presenting a previously undescribed cutaneous sign of Cushing's syndrome and review the relationship between Cushing's syndrome and the skin.

CASE REPORT

A 59-year-old Caucasian housewife gave a 2-year history of pain on walking associated with a loss of tissue substance from the heel pad and toe pulps. For the previous 8 years a similar process had affected the hands causing a progressive loss of substance from the finger pulps resulting in difficulty in fine manipulation. For many years she had noticed that her skin bruised easily, was fragile and healed poorly. Eight years previously a minor laceration of the right calf had failed to heal and required a skin graft. Two years before presentation, collapse of the left femoral head had necessitated a total hip replacement at another hospital. At that time treatment for osteoporosis with calcium and vitamin D, nandrolone decanoate 50 mg every third week, and Prempak C 0.625 (oestrogen 0.625 mg daily, norgestrol 0.15 mg daily days 17–28 of menstrual cycle) had been instituted.

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Examination revealed a generally well looking woman with a rather plethoric face. Erythrosis interfollicularis colli was noted, but there was no suggestion of moon-shaped facies or abnormal body fat distribution. The skin was thin and transparent, particularly over the anterior thighs and shins where the subcutaneous fat was visible through the thinned epidermis and where there was brown haemosiderin pigmentation. Elsewhere there were numerous fine scars and ecchymoses. The most marked change however was a dramatic loss of substance from the finger pulps (Fig. 1), heels and toe pulps. This was not tender and there was no associated epidermal change. There had been no previous suggestion of inflammation, pain, swelling or erythema. The patient was normotensive, the fundi and visual fields were normal and on general examination the only abnormality was a mild proximal weakness evident on rising from a crouched position.

Full blood count, serum electrolytes and liver function tests, fasting blood sugar levels, thyroid function tests, skull X-ray and examination of the urine were normal. Chest X-ray showed slight demineralization of the bones with normal lung fields and hand X-rays (Fig. 2) showed no evidence of boney reabsorbtion associated with the abnormal finger pulps. Plasma and urinary cortisol levels were elevated (09:00 441 nmol/l, NR 160–700 nmol/l; 24:00 414 nmol/l, NR < 220 nmol/l; urine free cortisol 2348 mmol/24 h, NR < 150 mmol/24 h) and were not suppressed with dexamethasone. Plasma ACTH was unrecordable both before and after dexamethasone. An abdominal CT scan showed a well defined mass in the left adrenal, 3·5 cm in diameter. Selenium cholesterol isotope scanning of the adrenal glands confirmed the functional nature of this mass, showing marked uptake on the left, with suppression of uptake on the right. At operation a yellow lobulated tumour was excised together with the left adrenal gland. The histology confirmed that it was a benign adrenal adenoma which was encapsulated and consisted of mixed foamy and dark cells, arising from an atrophic adrenal gland.

Prior to the operation, two skin biopsies were obtained under local anaesthesia. The biopsy from the right thigh showed abnormal collagen in the mid and lower dermis with a basophilic

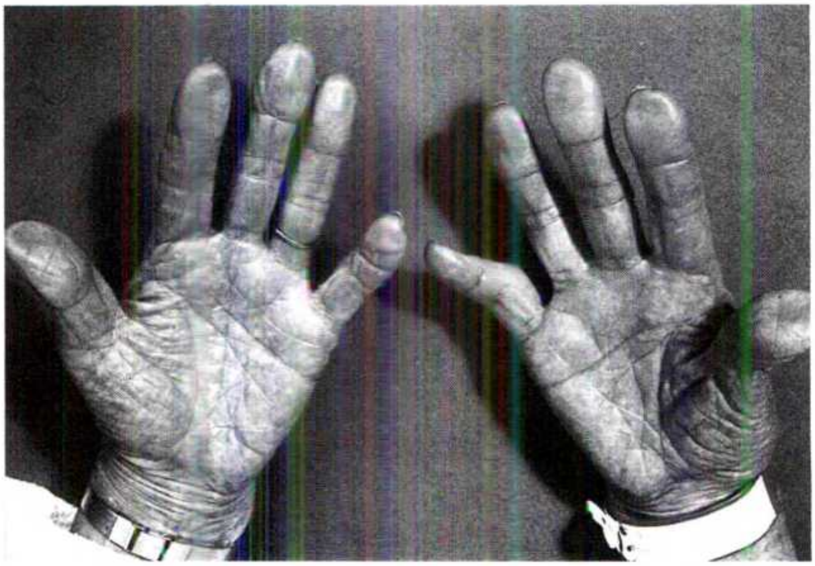

FIGURE 1. Loss of tissue substance from the finger pulps. Similar changes were also seen in the toe pulps.

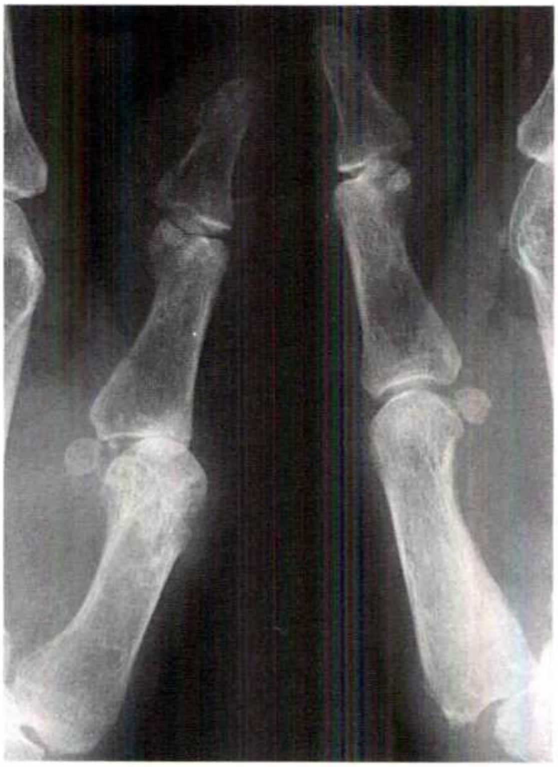

FIGURE 2. Lateral X-ray of the thumbs showing no evidence of boney reabsorption associated with the pulp abnormality.

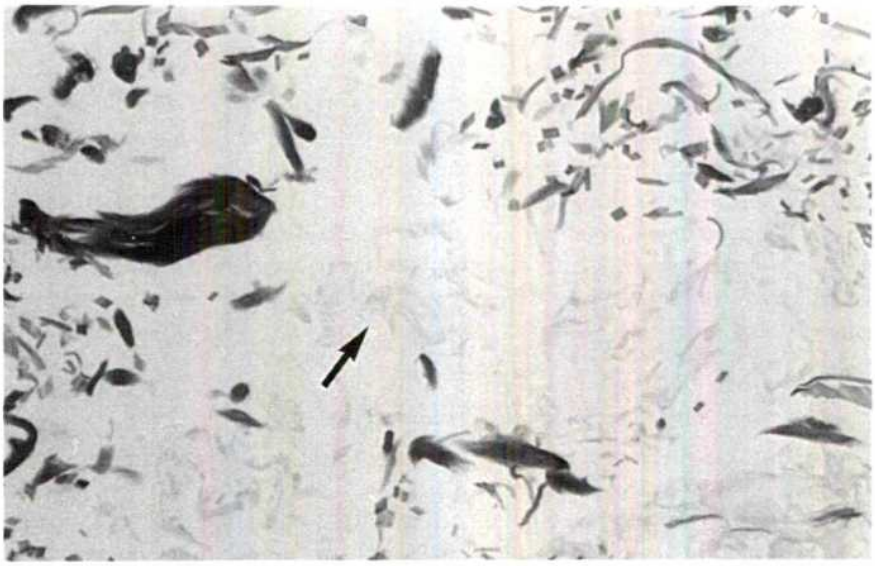

FIGURE 3. Skin biopsy of right thigh. Note lack of staining of central collagen fibres (arrow). Elastic–Van Gieson stain, × 100.

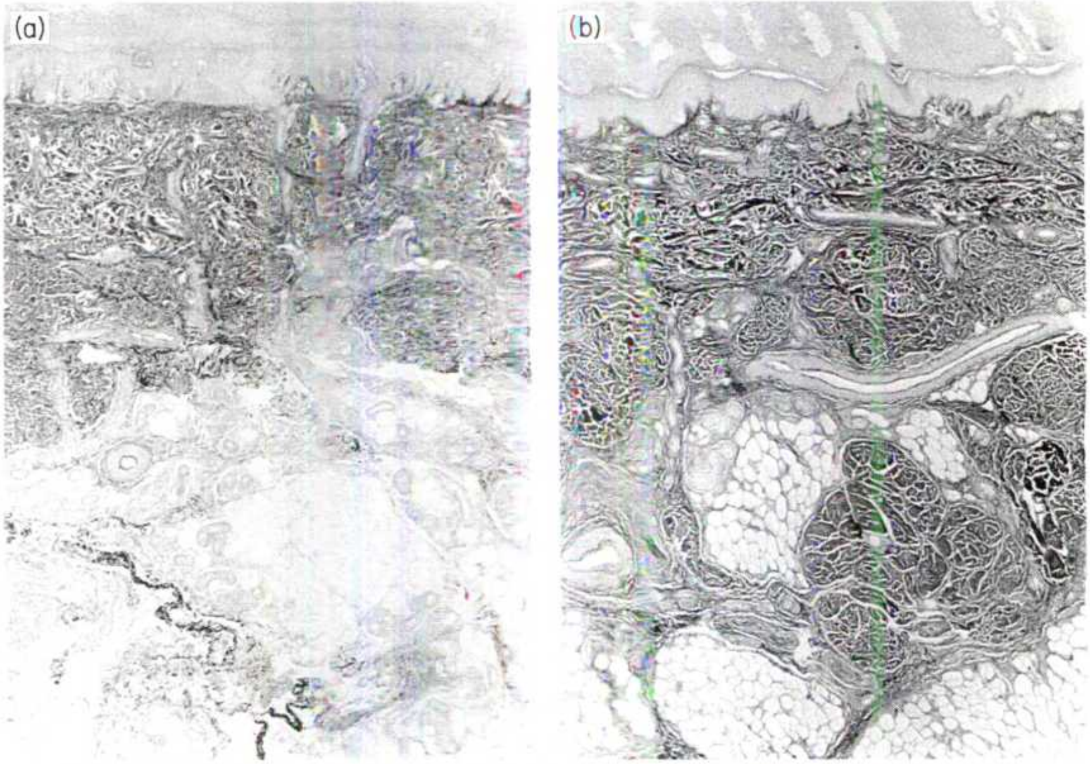

FIGURE 4. (a) Skin biopsy of patient's right great toe pulp. (b) Normal control. There is loss of dermal collagen with clumping of elastic fibres in the lower dermis and deep septae in the patient's biopsy. Both Elastic–Van Gieson stain, ×8.

hue and spindly texture similar to changes previously reported.[2] This collagen did not stain magenta with Elastic–Van Geison stain (Fig. 3) suggesting that it was immature or otherwise abnormal. A biopsy from the right great toe pulp (Fig. 4a) showed decreased amounts of qualitatively normal looking collagen with obvious dermal thinning when compared with tissue obtained from a normal post-mortem control (Fig. 4b), and there was evidence of elastin condensation both in the lower dermis and in the septae between fat lobules. The subcutaneous fat appeared normal and there was no evidence in either biopsy of an inflammatory cell infiltrate. Dermal elastin fibres, although clumped due to collagen loss, were otherwise normal.

DISCUSSION

Although cutaneous manifestations of Cushing's syndrome are well recognized, we are unaware of any case showing such marked loss of subcutaneous tissue of the extremities. Our biopsies suggest that the signs in our case are due to both qualitative and quantitative changes in dermal collagen, and should be regarded as a severe variant of the known cutaneous atrophy of Cushing's syndrome.[3] The histological changes seen in the thigh biopsy and the loss of toe pulp collagen presumably reflect steroid-induced derangement of fibroblast function of long duration. There is considerable experimental evidence to show that glucocorticoids have an inhibitory action not only upon fibroblast growth but also on collagen synthesis.[4]

The clinical features of patients with Cushing's syndrome have been reviewed in several large series[5–7] and it is clear that a careful assessment of the skin can provide useful information in the differential diagnosis of patients with obesity or with otherwise undifferentiated hypercortisolism. Bruising, unprovoked or with minimum trauma, was of high discriminatory value in the differential diagnosis of Cushing's syndrome from simple obesity, being 10 times more common in the former.[6] Myopathy, hirsuties, striae and plethora were also of differential diagnostic significance. Bertagna and Orth[7] showed that skin thinning was present in all of their patients with adrenal adenoma, but in only 11% of patients with adrenal carcinoma. It seems likely that the long-term, moderately raised levels of cortisol associated with adenomas have more potential to cause cutaneous changes than do carcinomas which present early with other features. It was also clear that signs of androgen excess, such as hirsuties, were much more common in adrenal carcinoma, reflecting the generally poorly differentiated nature of these tumours.

There is a striking clinical similarity between our patient and that reported recently by Fischer, Page and Hanna.[8] In their patient there was preceding inflammation leading to elastin destruction and focal cutis laxa of the finger and toe pulps. In our case, however, there was no historical, clinical or histological evidence of inflammation and elastin fibres, though clumped due to collagen loss, were otherwise normal. We suggest that all patients presenting with this clinical picture should have a skin biopsy for elastin stains and serum cortisol estimations to exclude Cushing's syndrome.

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Society Proceedings

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SUMMARIES OF PAPERS

Confocal laser immunofluorescence microscopy: a future tool in the differential diagnosis of subepidermal (immuno)bullous dermatoses

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Immunofluorescence microscopy (IFM) of perilesional skin has contributed substantially to the differential diagnosis of immunobullous dermatoses (IBD). Difficulties may arise in cases of subepidermal IBD characterized by *in vivo* binding of IgG in a linear pattern at the epidermal basement membrane zone (EBMZ). In these cases the level of *in vivo* bound IgG in the EBMZ has to be established, rather than the level of split formation. The use of EBMZ epitope markers (anti-BPag, anti-laminin, anti-collagen type-IV and type-VII) combined with double-wavelength IFM, provides an excellent means to assess the split level, but is a tricky method to use to establish the spatial distribution of IgG epitopes in the EBMZ.[1] Problems arise from technical limitations (resolution) of the optical system relative to the dimension of the EMBZ and technical artefacts ('optical shift' and 'colour shift') resulting from double-recording procedures and insufficiently selective optical channels. Both colour and optical shift may lead to erroneous conclusions as to the spatial relation between green- and red-stained epitopes in the EBMZ.

Confocal laser IF microscopy (CLM/IF) together with computer-aided image analysis would seem the method of choice to circumvent these problems.[2] In the present study unfixed cryostat sections of normal human skin were processed for double-fluorescent immunolabelling of the various EBMZ epitopes and analysed by a 'stage scanning' as well as 'beam scanning' CLM system. The findings can be summarized as follows:

(a) successful multiparameter analysis by CLM/IF of spatially related EBMZ epitopes is largely dependent on the selectivity of optical wavelength channels and the possibility to prevent optical shifts;

(b) the fulfilment of these conditions differ with the type of CLM system explored;

(c) computer-aided image analysis circumvents the problem of colour shifts and opens new ways to use the overlap of green and red fluorescent colours as a valid source of information.

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Immune deposits in the skin of healthy children

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Knowledge about the occurrence of immune deposits at the dermo-epidermal junction (DEJ) in the skin of healthy individuals is essential in determining the diagnostic value of immunofluorescence microscopy (IFM) in autoimmune connective tissue disorders. Few studies have focused on this issue in adults.[1-3] Control IFM data pertaining to the skin of healthy children are still lacking. The present study attempts to fill this gap and forms part of a broader study in children showing clinical symptoms possibly mediated by (auto)immune complexes.

A total of 21 healthy children (1–14 years; 19 male and two female) participated in this study with the informed consent of their parents. Biopsy specimens were obtained from both light-exposed (wrist, $n = 20$) and light-protected skin (abdominal region, $n = 21$). Unfixed cryostat sections were processed for routine IFM, using conjugated antibodies against IgG, IgM, IgA, C3c and fibrin(ogen). The findings can be summarized as follows: Healthy children showed a higher incidence of IgM deposits at the DEJ of light-exposed skin as compared to healthy adults.[3] This was most evident in children less than 3 years old ($P < 0{\cdot}05$). The IgM deposits at the DEJ were found more often in light-exposed (75%) than in light-protected skin (14%). The IF patterns in light-exposed skin ranged from granular (55%) to homogeneous/tubular (40%) and fibrillar (10%). In most cases, the IgM patterns were discontinuous and showed weak (1 +) to moderate (2 +) fluorescence. C3c deposits were found at the DEJ in 25% of light-exposed skin specimens. Their presence coincided with granular but not with homogeneous IgM deposits. In one apparently healthy case we found distinct granular IgM, IgA, IgG and C3c deposits at the DEJ in light-exposed but not in light-protected skin. Although this profile was suspect for lupus erythematosus, other relevant criteria proved incompatible with this diagnosis. Subepidermal capillary walls in light-exposed skin occasionally showed granular fluorescence for IgM and C3c. Epidermal *in vivo* ANA was consistently negative.

From a diagnostic point of view our findings show the need for careful interpretation of Ig(M) deposits at the DEJ, particularly in children less than 3 years old.

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Atopic dermatitis: methodology of patch tests with house dust mite allergens

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Positive patch tests with allergens occur in patients with atopic dermatitis (AD)[1-3] and the methodology used varies widely. We performed patch tests with house dust mite (HDM) allergens, studying the following variables: allergen concentration, application time, intensity of tape-stripping.

In 20 patients with AD and a positive intracutaneous test and RAST for HDM, patch tests were performed with HDM solutions provided by Laboratory Diephuis (LD), Groningen and HAL Allergen Laboratory (HAL), Haarlem, containing 20 ×, 100 × and 500 × the standard concentration used for the intracutaneous test. Purified HDM antigen: 10 and 50 μg/ml P1 Ag solution (Allergology Laboratory, University Hospital, Groningen) and as controls LD and HAL solution fluid and a blank patch test were used. Silver patches with a square Whatman filtration paper 1 × 1 cm for 0·03 ml solution and fixation with hypoallergenic tape were used. The series of 11 patch tests was applied four times, on stripped and non-stripped clinically normal skin on the back for 24 and 48 h, and read at 24, 48, 72 and 96 h. Two methods of tape-stripping were used: A, rigorous stripping (15 × ; $n = 7$); B, moderate stripping (8 × ; $n = 18$). In 5/7 Group A patients the patch tests were repeated using method B.

Non-specific reactions due to stripping, tape fixation or patch test occlusion were observed in many patients, especially after rigorous stripping and 48 h application: Group A, after 24 h application 3/7 (43%), after 48 h application 6/7 (86%); Group B: after 24 h application 2/18 (11%), after 48 h application 7/18 (39%).

Repetition of the patch tests using method B diminished the number of patients with non-specific reactions due to stripping from 4/5 to 1/5. Because of the frequency of non-specific reactions, it was difficult to be certain of supposedly specific reactions. Moreover, a simple and reliable *in vitro* control test clinically relevant for AD is lacking, making it impossible to decide as to the necessity of stripping.

Specific reactions appeared to occur more frequently by testing with the highest concentration of the HDM solutions, after 48 h application time, after rigorous tape-stripping and in patients with an IgE serum level > 1000 kU/L. The latter was related to a positive history of respiratory atopy. In two patients supposedly specific reactions were reproducible.

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Development of a highly vapour-permeable wound dressing

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Recently we have developed a new occlusive wound dressing consisting of a highly vapour-permeable film, which does not cause the usual problems of retention of wound fluid.[1] This film was developed as a fluid-regulating top layer for artificial skin, but alone was found to be an effective dressing. The new film consists of a 0·015 mm thin, elastic polyetherurethane (PEU) membrane that is hydrophilic and semi-permeable. Water cannot leak through it, nor can bacteria penetrate it, but water vapour can diffuse through at a high rate (water vapour permeance: PEU dressing 20·1, OpSite® 5·3, Duoderm/Granuflex® 1·7 g.m.$^{-2}$.h^{-1}.kPa^{-1}).[2] As a result, superfluous wound fluid diffuses through the dressing and the film adheres without additional adhesive backing to the retained coagulated exudate.

Histo-morphometrical evaluation of the healing of partial-thickness wounds in guinea-pigs showed that epithelialization with PEU dressing was 25% faster compared to OpSite® and 150% faster compared to gauze-dressed wounds.[3] The enhanced epithelialization rate might be explained by the gelatinous coagulum retained under the PEU dressing. Scanning electron microscopy and immunohistochemistry revealed that the coagulum consists of a fibrillar mesh of fibrin and fibronectin molecules that is used by epidermal cells for adherence and spreading during migration.[4] Under OpSite® the exudate was more watery and not coagulated into a fibrillar mesh. The enhanced healing rate might also be explained by an increased concentration of growth factors present in the condensed exudate under PEU dressing.

Clinical application on donor sites showed that the PEU dressing indeed prevented fluid retention and led to considerably less wound pain.[5] The PEU dressing remains on the wound for 5 days and can easily be peeled from coagulum without disrupting the vulnerable epidermis. Since the PEU dressing adheres to wet wound surfaces only and not to intact skin, it can be especially useful on erosive lesions in patients with bullous disease (e.g. epidermolysis bullosa), since the fragile skin is not damaged while removing the PEU dressing.

The PEU dressing is expected to become available on the market in the near future. Further research activities are aimed at the development of a complete artificial skin combining the PEU dressing as top layer with a microporous, biodegradable, synthetic bottom layer.

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Acrodermatitis chronica atrophicans

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Ten patients (eight women, two men) with acrodermatitis chronica atrophicans (ACA) are presented. Diagnosis was based on the clinical picture, demonstration of spirochaetes in the affected skin[1] and positive serology against *Borrelia burgdorferi* (IgG$\geq$1/64) using an immunofluorescence test. ACA had been present for 1·5–18 years (mean 4·5 years). ACA was preceded by a recognized tick bite in two cases and erythema chronicum migrans in three cases. Two patients had pronounced skin atrophy and one patient had fibrotic nodules located around the ankle and on the pretibial area. The histology of a fibrotic nodule showed the presence of spirochaetes located in an extensive lymphoplasmacellular infiltrate and hyalin fibrosis.[1] The remaining patients showed a varying degree of bluish-red discoloration and slight infiltration. Heat intolerance of affected skin was a marked complaint in this early stage of ACA. The diagnosis of the early stage of ACA is easily missed. Venous insufficiency was the initial diagnosis in four patients, arthrosis in two patients and polyneuropathy in one patient. Seven patients were treated with oral phenoxymethylpenicillin 500 mg four times a day for 4 weeks and three patients with oral doxycycline 100 mg twice daily for 4 weeks.

Serological follow-up performed after 6 months in five patients was negative in four of the patients and showed no change in one.

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CASE REPORTS

Epidermolysis bullosa acquisita

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History. Male, aged 41 years. Since 1976 he has had periodic pretibial bullae following minor mechanical trauma. The mucosae were never involved. There was no familial history. A diagnosis was made in 1976 of lichen ruber pemphigoides because of multiple subepidermal

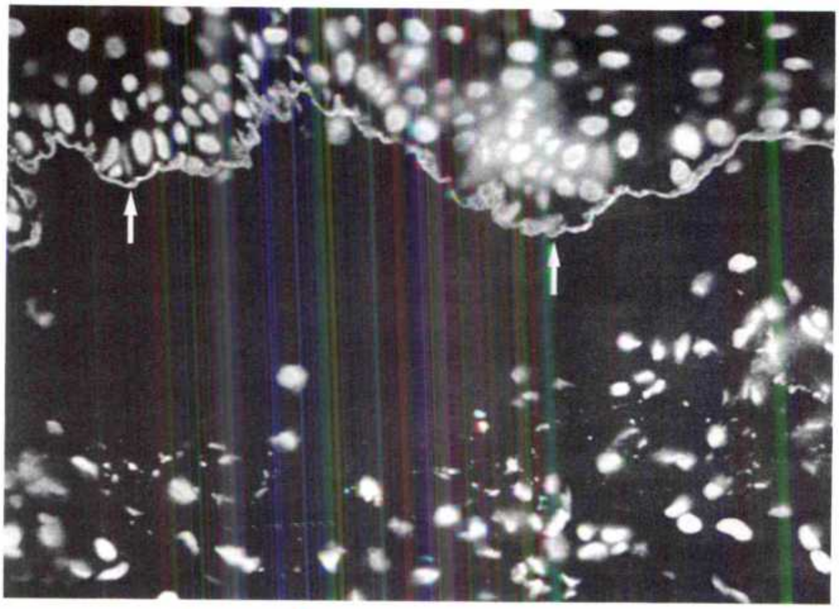

FIGURE 1. Staining of collagen type VII with LH7.2 monoclonal antibody in the roof of a subepidermal blister (arrow).

IgM-positive colloid bodies and linear IgG deposition at the epidermal basement membrane zone (EBMZ). Treatment with local and oral corticosteroids (20 mg daily), vitamin A-acid, etretinate and dapsone was not effective. Nail dystrophy developed in 1979. An exacerbation occurred with mechanically-induced bullae in 1988 with a common cold infection.

Examination. Tense bullae with surrounding erythema and erosive lesions were present on the chin, nostril, nipple, dorsum of two fingers and shins. Nikolsky's sign was negative. There were pretibial scars with milia. All the nails were dystrophic.

Histology. This showed a subepidermal split with normal epidermis as the roof.

Immunofluorescence. Perilesional skin, lower leg. At the EBMZ there was a continuous deposition of IgG in a linear, ragged pattern, granular and linear C3c and a focal deposition of fibrin. There were no deposits of IgM and IgA. EBMZ epitope mapping showed the presence of bullous pemphigoid antigen, laminin and collagen type IV and type VII in the roof of the bulla (Fig. 1).

Normal skin, extensor surface forearm. There was a linear, but ragged pattern of IgG at the EBMZ.

Serum: possible presence of IgG (1:10) at the BMZ of monkey oesophagus and human mucosa.

Electron microscopy. Normal skin: there were abnormal deposits beneath the lamina densa zone, but a normal structure and number of anchoring fibrils and other structures of the EBMZ.

Perilesional skin: the lamina densa was not present in the floor of the bulla, and the roof was absent due to tissue processing.

Immunoelectron microscopy. Normal skin: immunoperoxidase labelling of cryostat sections showed deposits of IgG below the lamina densa at sites of anchoring plaques and separated from the lamina densa by an electron-lucent zone. The lamina lucida and hemidesmosomes showed no deposits of IgG.

Treatment. The erosions were dressed with gauze but repeated dressings impaired epithelialization. The use of a vapour-permeable poly-etherurethane wound dressing lacking an adhesive backing[2] led to prompt healing. No systemic treatment was necessary.

Discussion. Epidermolysis bullosa acquisita is an autoimmune bullous skin disorder. With epitope mapping and the demonstration of collagen type VII in the roof of a blister, it was shown that the subepidermal bulla formation occurred below the lamina densa. Immunoelectron microscopy of ultrathin cryosections showed deposits of IgG beneath the lamina densa as well as at the sites of anchoring plaques. These findings are those of epidermolysis bullosa acquisita rather than other bullous dermatoses that show linear immunoreactants at the EBMZ.[2]

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Pemphigoid gestationis

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History. Female aged 35 years of Creole descent. On the fourth day following the birth of her fifth child, a daughter, by Caesarian section, she developed itchy lesions around the umbilicus which after 1 week became bullous.

Examination. Multiple urticarial papules on the abdomen, upper legs, arms, hands and face with vesicles at their borders and tense bullae in the centre of some lesions.

Histology. Lesion on arm. There was subepidermal bulla formation with oedema of the upper dermis and a perivascular infiltrate that consisted of lymphocytes and many eosinophils.

Immunofluorescence. Perilesional skin, arm. There was a linear band of C3c at the epidermal basement membrane with no deposits of IgG (Fig. 1). The normal skin on the buttocks showed an identical pattern with a linear deposition of C3c.

Circulating IgG-class anti-BMZ antibodies were detected using human skin in a titre of 1 : 20, but not on monkey oesophagus. By the C3-fixation test, complement deposition was found at the BMZ of human skin and monkey oesophagus after incubation with the patient's serum and the fresh serum of a control, demonstrating the presence of the pemphigoid gestationis (PG) serum factor.

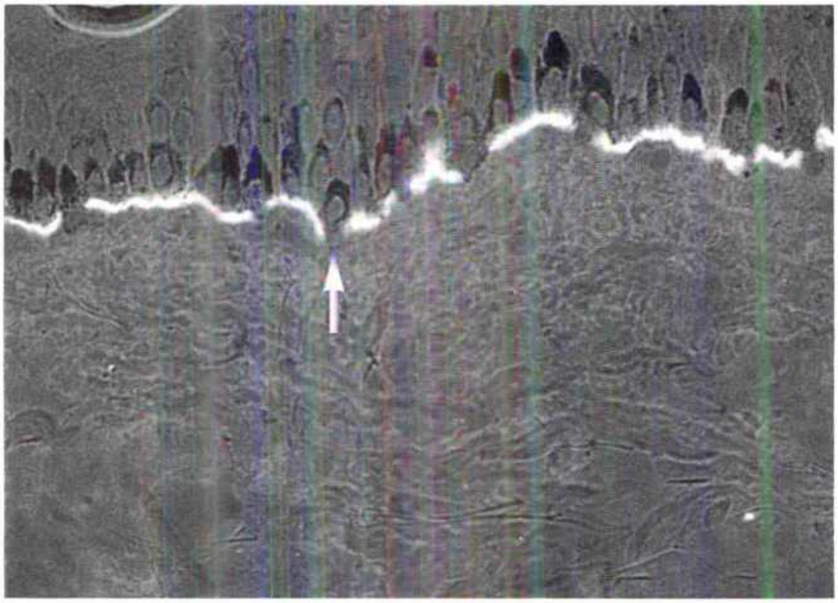

FIGURE 1. Immunofluorescent staining for C3 in perilesional skin. The combined use of transmitted light shows the staining that is interrupted at the site of probable melanocytes (arrow).

Laboratory investigations. IgE 1026 kU/1, TPHA 1:320. Negative tests for HLA-antigens HLA-B8, -DR3 and -DR4.

Treatment. A complete clinical remission was produced with oral prednisolone initially at a dosage of 40 mg daily. The serum IgE level dropped to 347 kU/l and the TPHA became negative. After 18 months C3c deposition could no longer be demonstrated in the skin. The patient developed some itching premenstrually and this occurred with an oral contraceptive that had to be stopped after a few days. The child never had any skin lesions.

Discussion. So far only four Negro women with pemphigoid gestationis have been described.[1] The condition usually begins in the second or third trimester of pregnancy and occasionally post-partum. The severity of the symptoms increases with subsequent pregnancies. A bullous eruption has been noted in 10% of the babies. The blister formation occurs high in the lamina lucida and linear deposition of C3 at the EBMZ is found in 100% of patients and IgG in 25%. The PG antigen has been characterized as a 180 kDa glycoprotein that shares determinants with the 220 kDa bullous pemphigoid antigen.[2]

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Junctional epidermolysis bullosa letalis

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History. Female, aged 3 days, second child of consanguinous parents. At birth several fingers and toes showed onycholysis. Bullae developed 1 day later, especially on pressure sites. A brother of the father with a similar bullous skin disorder died at the age of 3 months. The older sibling of the patient was a healthy boy.

Examination. Multiple small and large bullae were seen, especially on pressure sites, some tense and intact, others leaving eroded areas. The Nikolsky's sign was negative. Almost all the fingers and toes showed onycholysis. In the oral cavity some small bullae were noted.

Histology. A biopsy from perilesional skin showed subepidermal bulla formation.

Immunofluorescence. Epitope mapping of the EBMZ in perilesional skin showed bullous pemphigoid antigen and traces of laminin in the roof of small subepidermal bullae. Bright staining for laminin was observed in the floor of these bullae indicating the split formation high within the lamina lucida (Fig. 1).

Electron microscopy. A biopsy from perilesional skin confirmed the site of bulla formation to be within the lamina lucida (Fig. 2). A decreased number of hemidesmosomes was found in the roof of the bulla and, to a lesser degree, in intact skin. In the blister cavity degenerated anchoring filaments were observed. The anchoring fibrils in the dermis were intact.

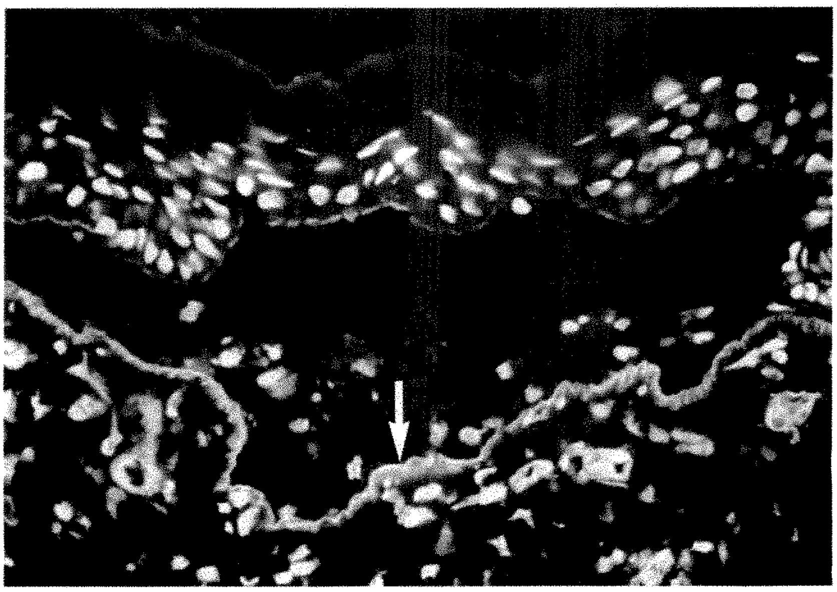

FIGURE 1. Immunofluorescent staining of laminin (BM marker) in perilesional skin, showing subepidermal bulla formation. Bright staining for laminin is observed in the floor (arrow) and weak, patchy staining in the roof of the bulla.

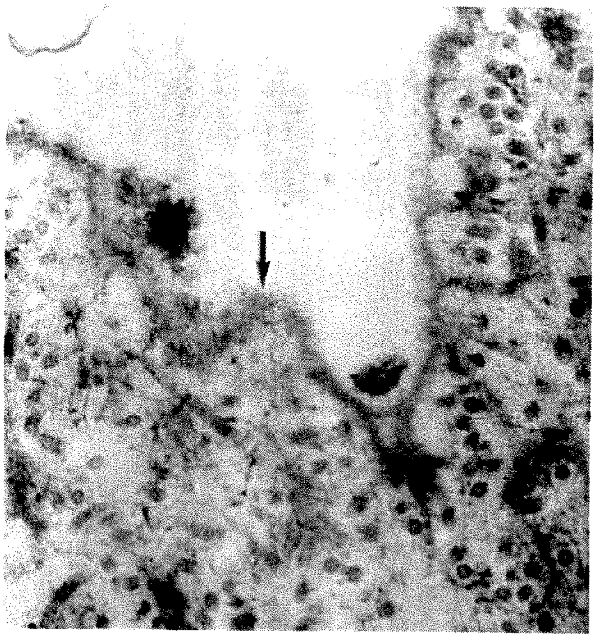

FIGURE 2. Electron micrograph of lamina densa (arrow) at the floor of a bulla.

Treatment. The patient was nursed and treated according to the protocol of the Dutch Genodermatoses Study Group. The baby failed to thrive and died at the age of 4 months. The parents would not accept future prenatal diagnosis because of religious reasons.

Discussion. The genodermatosis epidermolysis bullosa (EB) is known to consist of three main groups: epidermolytic, junctional and dermolytic EB. Bulla formation in junctional EB occurs within the lamina lucida as can be demonstrated by EBMZ epitope mapping. Six variants of junctional EB have been described, of which the letalis or EB atrophicans generalisata gravis (Herlitz) variant is the most severe. Almost all children with this variant appear to develop perioral and perinasal persistent lesions after the age of 3–5 months and die before the age of 2 years.[1] Bulla formation is related to abnormal hemidesmosomes.[2] Treatment with phenytoin is not effective in junctional EB letalis.[3] Prenatal diagnosis using Mab GB3 is possible.[2]

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Porokeratosis disseminata with cutaneous amyloidosis

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History. Male, aged 73 years. From the age of 67 years he gradually developed a disseminating, slightly itchy skin eruption. There was no family history of the condition or any relation to actinic exposure.

Examination. A bilateral, symmetrical eruption was seen on legs and buttocks, consisting of numerous red-brown, flat, hyperkeratotic papules (Fig. 1), forming confluent hyperkeratotic plaques with fissuring on the buttocks. A raised border could be discerned with difficulty in rare papular lesions. The palms and soles were normal.

Histology. Biopsies from the legs and buttocks showed acanthosis and hyperkeratosis and multiple parakeratotic columns (cornoid lamellae) in each lesion. At the base of these columns the granular layer was lacking and dyskeratosis was observed. A sparse dermal, mainly perivascular, lymphocytic infiltrate was seen. In the papillary dermis multiple Congo-red positive bodies were noted.

Immunofluorescence. Biopsies from the legs and buttocks showed discontinuous, homogeneous fibrin and granular C3 deposition at the EBMZ. In the papillary dermis multiple IgM colloid bodies were noted, which also stained positive for cytokeratin (pan cytokeratin antibody) and SAP (serum amyloid P component), but negative for amyloid-A (Fig. 2).

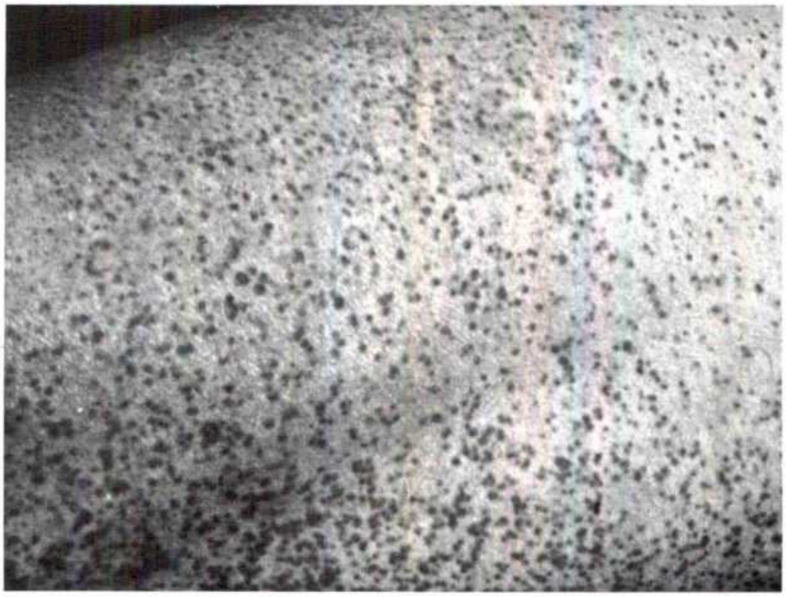

FIGURE 1. Disseminated red-brown hyperkeratotic papules on the upper leg.

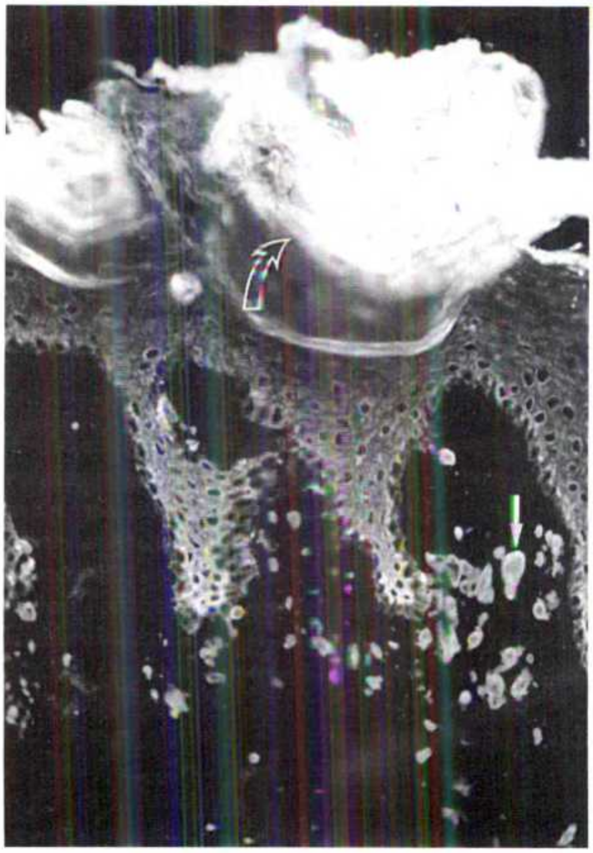

FIGURE 2. Double immunofluorescent staining of IgM and cytokeratin in lesional skin, demonstrating IgM⁺ KIF bodies in the papillary dermis (arrow) at the site of a brightly fluorescent cornoid lamellum (open arrow).

Treatment. Etretinate, 0·5 mg/kg/day, made the hyperkeratotic fissuring worse and topical fatty ointments were preferred by the patient. Carcinomatous change in the lesions was not observed.

Discussion. A case similar to this disseminated bilateral hyperkeratotic papular variant of porokeratosis, with histology showing multiple cornoid lamellae has been reported.[1] In the present case the formation of multiple IgM^+ and SAP^+ keratin intermediate filament (KIF) bodies, presumably derived from apoptotic keratinocytes,[2,3] was noted. This may be inherent to the pronounced keratinization disorder in this variant of porokeratosis. Amyloid P protein, binding to KIF bodies,[3] may originate from the serum or be produced by basal keratinocytes.

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Annular elastolytic giant cell granuloma

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History. Male, aged 54 years. He had non-itchy skin lesions from 3 months of age. Treatment with betamethasone diproprionate cream for 6 weeks did not result in any improvement.

Examination. The back and the extensor side of the arms showed red-brown macular and papular lesions. A slightly elevated annular border with slight atrophy in the centre was noted in some macular lesions. On the scalp two livid-red, partly yellow-brown macular lesions with atrophy were observed.

Histology. Biopsies from the border of annular lesions on the arms and back showed a granulomatous infiltrate in the upper and mid-dermis consisting of lymphocytes, histiocytes and numerous multinucleate giant cells. Necrobiosis and actinic elastosis were not observed. The Verhoeff stain demonstrated an absence of elastic fibres at the site of the granulomatous infiltrate. Engulfed elastic fibres were frequently found in giant cells.

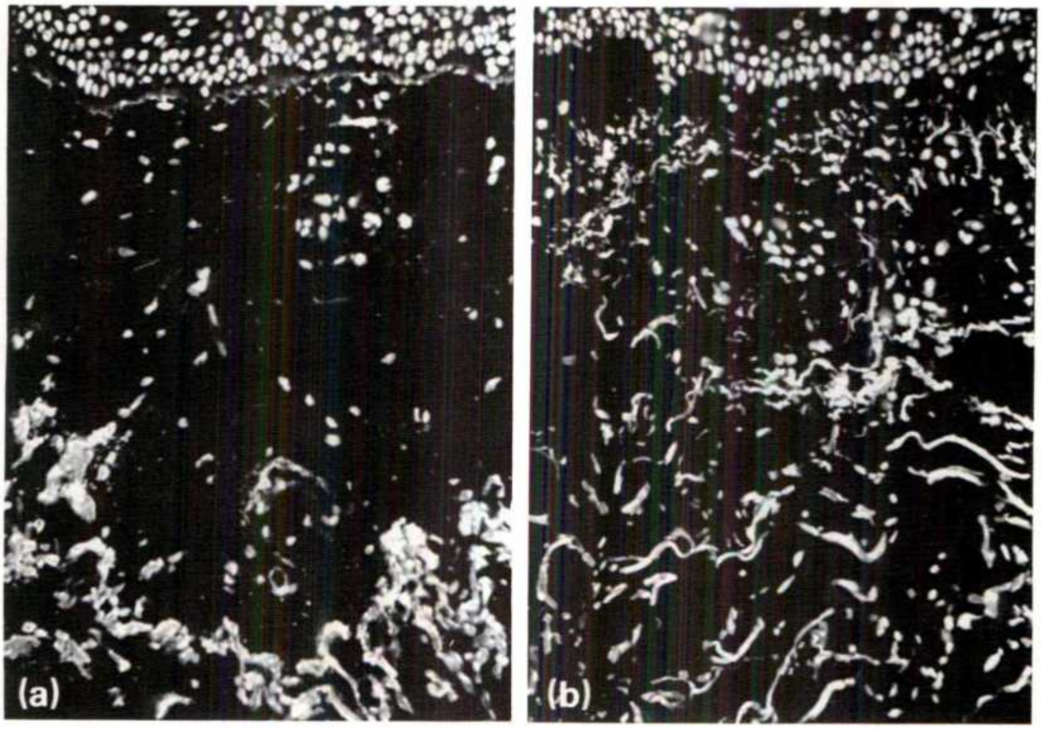

FIGURE 1. Immunofluorescent staining of SAP (serum amyloid P). (a) Lesional skin: absence of SAP$^+$ elastic fibres in the upper dermis and clumpy staining of mid-dermal elastic fibres. (b) Uninvolved skin: normal staining pattern for SAP$^+$ fibres.

Immunofluorescence. Lesion, arm, anti-serum amyloid P (SAP) component staining.[1] In the upper dermis at the site of the granulomatous infiltrate there was an absence of SAP$^+$ elastic fibres (Fig. 1a). Clinically uninvolved skin: normal SAP$^+$ elastic fibre pattern (Fig. 1b).

Treatment. Spontaneous regression occurred within 3 months.

Discussion. In 1975 O'Brien described giant cell elastophagocytosis in lesions on sun-exposed skin, which he thought to be induced by actinic damage.[2] In 1979 lesions with a similar histology but without actinic elastosis were described as annular elastolytic giant cell granuloma (AEGCG).[3] Similar lesions have been described as atypical necrobiosis lipoidica and Miescher's granuloma of the face. Elastophagocytosis has also been observed in granuloma annulare and other granulomatous inflammatory processes in the skin and may be a non-specific reaction pattern.[4,5] Necrobiosis, described as absent in AEGCG, can also be absent in granuloma annulare,[4] which suggests that AEGCG and granuloma annulare are part of the same disease spectrum.

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Anetoderma of Jadassohn–Pellizari

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History. Female, aged 37 years. Since 1980 she suffered from red, itchy skin lesions, at first slightly elevated but later showing flattening. The family history was negative.

Examination. On the upper part of the thorax, arms, neck and face there were round and oval, erythematous or light-brown lesions. Some were slightly elevated and others were slightly depressed with a wrinkled surface (Fig. 1).

Histology. Erythematous lesion on thorax. There was a dermal perivascular infiltrate consisting of neutrophils and eosinophils and mononuclear cells.

Atrophic lesion on thorax. There was a dermal perivascular infiltrate with some neutrophils and eosinophils. The elastic fibres were absent in the upper part of the dermis.

The uninvolved skin showed no abnormality.

Immunofluorescence. Erythematous lesion on arm. Staining with anti-serum amyloid P (SAP)[1] showed that at the site of the inflammatory infiltrate SAP$^+$ elastic fibres were absent.

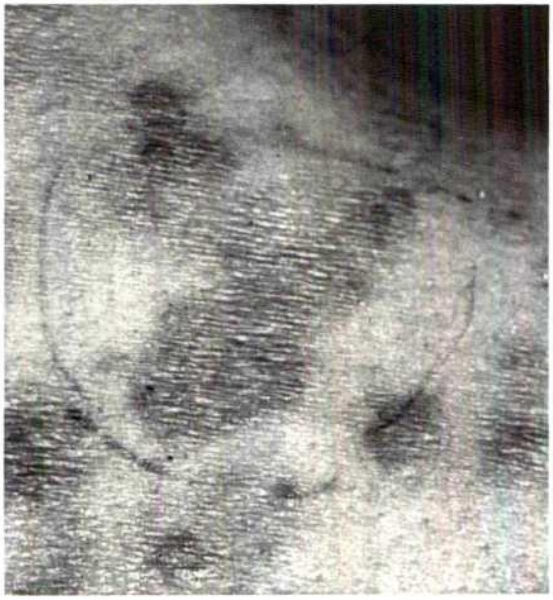

FIGURE 1. Wrinkled appearance of the erythematous shoulder lesions.

Atrophic lesion on thorax. The SAP$^+$ elastic fibres were absent.
Clinically uninvolved skin. Normal SAP$^+$ elastic fibre pattern.

Laboratory investigations. The ANA was 1:80 homogeneous staining.

Treatment. Topical steroids and treatment with hydroxychloroquine were without any significant effect.

Discussion. Anetoderma, of which there is an inflammatory (Jadassohn–Pellizari) and a non-inflammatory (Schweninger–Buzzi) variant, is characterized by local loss of the dermal elastic fibres.[2] This could be due to decreased synthesis of elastin and/or increased elastinolysis. Neutrophilic granulocytes produce elastinolytic enzymes.[3] In cutis laxa, which may be part of the same spectrum of disease as anetoderma,[4] the abnormalities are more extensive.

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Cutis laxa acquisita: the inflammatory variant

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History. Female, aged 25 years. The skin disorder started 3 years earlier with itchy lesions on the hands and lower arms followed by more generalized urticarial lesions, persistent facial oedema and progressive increased wrinkling of face, neck and trunk. There was no family history.

Examination. The patient looked much older than her actual age. Marked wrinkling was seen at the corners of the mouth, on the neck and trunk and especially on the breasts (Fig. 1). The face, trunk and, to a lesser extent, the extremities showed numerous macular or urticarial erythematous lesions, up to 1 cm in diameter, sometimes with a vesicular appearance. On the lower arms and palms of the hands a few vesicles and lenticular erythematous lesions with mild central blistering were noted.

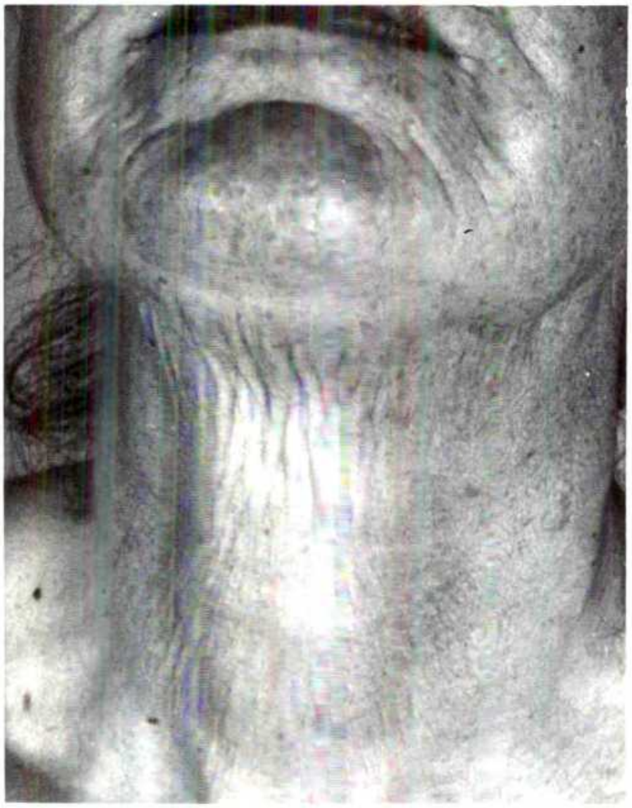

FIGURE 1. A 25-year-old woman with prematurely aged appearance and increased wrinkling in the neck.

Histology. A biopsy of a vesicular lesion on the palm showed oedema and micro-abcesses with neutrophilic granulocytes in the papillary dermis. There was a dense lower dermal infiltrate of mononuclear cells and eosinophils with a decreased amount of elastic fibres that showed an abnormal morphology (e.g. mouse tail formation), with the Verhoeff stain. A biopsy of an urticarial lesion from the back showed a similar picture, but without the oedema. Verhoeff staining of biopsies from atrophic skin and clinically uninvolved skin from the neck demonstrated an abnormal elastic fibre pattern and there was an absence of inflammatory infiltrate in both.

Immunofluorescence. Several biopsies taken from erythematous lesional, perilesional and normal skin showed no deposits of immunoglobulins. Perivascular deposits of fibrin and absence of SAP^+ (serum amyloid P component) elastic fibres at the site of inflammatory infiltrates were noted in the erythematous skin lesions.[1]

Electron microscopy. Biopsies from atrophic skin and clinically uninvolved skin from the back both showed abnormal elastic fibres.

Laboratory tests. Routine haematological and blood chemistry tests and urine examination gave normal values. ANA 1:10, homogeneous. Anti-Borrelia antibodies (IgG, IgM): negative. Total IgE 319 kU/l, RAST house dust mite: 2^+. Pulmonary function tests: slight expiratory obstruction, partly reversible, without progression 1 year later.

Treatment. Dapsone and hydroxychloroquine were without effect. A remission of the inflammatory component was finally achieved using prednisolone, initially 60 mg daily and later the dose being reduced, azathioprine 100 mg daily and clofazimine 100 mg daily. It was considered that the progression of premature ageing was stopped. A face-lift was later performed by a plastic surgeon.

Discussion. Cutis laxa is characterized by extensive lack of elastin fibres, probably due to defective synthesis as was demonstrated in our patient. There is also increased elastinolysis, due to an inflammatory factor with neutrophils producing elastinolytic enzymes.[2] Three hereditary forms are recognized: the life-threatening autosomal recessive variant with involvement of internal organs, a relatively benign autosomal dominant and an X-linked variant.[3] Our patient presented as a case of cutis laxa acquisita which might have been a new mutation of the autosomal dominant variant, which often starts after childhood. No more than 26 patients with cutis laxa acquisita have been described,[4] of whom about 50% had an inflammatory component. In our patient this appeared to further accelerate the premature ageing process, necessitating anti-inflammatory treatment.

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British Journal of Dermatology (1990) **123,** 691–694.

Correspondence

Acantholytic lesions in the vagina

SIR, We wish to report a case of isolated acantholytic lesions involving the vagina in a patient with a family history of Hailey–Hailey disease.

A 40-year-old woman was seen at the Department of Surgery in April 1988 with a history of severe dyspareunia with deep post-coital pain during the previous 1·5 years. In 1986 she had been treated with broad-spectrum antibiotics for pneumonia and this had resulted in transient oral and vaginal candidiasis, which required local antifungal therapy. The uterus was slightly enlarged and tender and there were white erosions in the posterior fornix. A total hysterectomy was performed which included the erosive lesions in the uppermost part of the vagina. On histology there were focal areas of acantholysis of the vaginal epithelium (Fig. 1) with some evidence of dyskeratosis in the deeper part of the epidermis. Following hysterectomy the severe dyspareunia disappeared completely. She had no skin lesions to suggest either Hailey–Hailey disease or Darier's disease, but there were whitish longitudinal streaks on the nail plates of both thumbs suggestive for Darier's disease.

Her mother had classical Hailey–Hailey disease with typical lesions involving the axillae, groins and the inframammary folds. The skin biopsy showed the histological features of Hailey–Hailey disease rather than Darier's disease. The patient's brother and four sisters were later examined and all were unaffected, apart from her youngest sister who had had Hailey–Hailey disease over the past 3 years affecting the groins and vulva. This diagnosis had been confirmed by a skin biopsy. Deep post-coital pain had occurred on occasions in this sister and suggested that there were periodic acantholytic lesions of the vaginal mucosa. Our patient had two children aged 13 and 16 and neither had any evidence of the disease.

The clinical features of our patient and her affected relatives are consistent with the diagnosis of Hailey–Hailey disease, as was their histology. However, our patient also had nail changes that are claimed to be specific for Darier's disease.[1] Lesions of the mucous membranes can occur in Darier's disease,[1-3] as well as in Hailey–Hailey disease.[4-6] The histological differentiation between the two disorders may be difficult in the mucosal lesions such as occurred in our case. The combined features of both disorders have also been

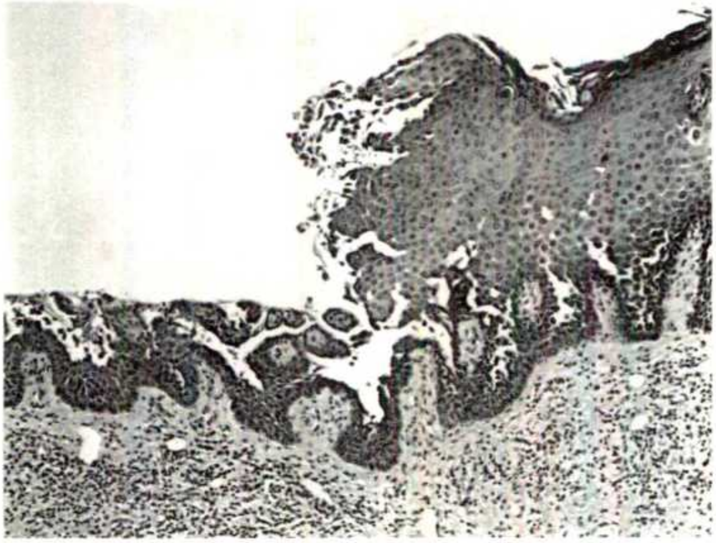

FIGURE 1. Acantholytic lesion of vaginal mucosa. Superficial layers of the squamous epithelium are partially missing. In the underlying connective tissue a reactive inflammatory response can be seen (H&E, × 75).

described.[7–10] Our case is similar to that reported of a patient with Hailey–Hailey disease with erosive lesions in the vagina causing severe dyspareunia.[6] In our patient the acantholysis may have been triggered off by trauma and by vaginal candidiasis. Typical lesions in familial pemphigus may be provoked by *Candida albicans*.[11]

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Pityriasis rubra pilaris in Indians

SIR, We read with surprise the letter by Sehgal *et al.*[1] The authors gave the impression that pityriasis rubra pilaris (PRP) is uncommon in racially pigmented skin and perhaps extremely rare in Indians. They based their conclusions on having seen only six cases among 350,000 out-patients during the past decade. However, in our department we see on average one or two cases per year of classical adult onset (Type I) PRP that fulfil clinical and histopathological criteria for its diagnosis. Though the classical juvenile onset (Type III) is uncommon, the atypical types (both juvenile and adult) as well as the circumscribed types are frequently encountered.

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Direct antiproliferative effect of cyclosporin A on cultured keratinocytes and fibroblasts

SIR, We were interested to read the recently published report[1] demonstrating a direct antiproliferative effect of cyclosporin A on cultured keratinocytes and fibroblasts. These authors observed a reversible, dose-dependent inhibitory effect and that the presence of serum attenuated this response. These findings were of interest to us because we previously observed the same phenomena and reported them in several earlier publications.[2,3] We cannot understand these citation omissions of our *in vitro* findings because they were properly cited by investigators confirming an *in vivo* direct antiproliferative effect on keratinocytes[4] and this reference was included in the bibliography of their paper.

The authors raised the question of the possible molecular mechanism by which cyclosporin A inhibits keratinocyte growth, and we suggest that it may exert its antiproliferative effect by decreasing the number of high affinity epidermal growth factor receptors.[5]

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Persistent bite reactions responsive to photochemotherapy

SIR, Cutaneous reactions to bites from arthropods are common and immediate reactions are pruritic, erythematous papules that resolve within an hour. Sometimes a delayed reaction occurs and these lesions are intensely pruritic and the intensity and duration of symptoms is variable and often correlates with the number of bites received.[1] We describe two cases of patients with persistent insect bite reactions who having failed standard therapy, were successfully treated with oral psoralens and long-wave ultraviolet light (PUVA).

Two middle-aged Caucasian men presented with multiple insect bites. Patient 1 noted multiple chiggers on his body on a hunting trip. Both men had pruritic, erythematous urticarial papules on their neck, trunk and buttocks. A biopsy from each patient revealed a superficial dermal perivascular lymphocytic infiltrate with eosinophils. Treatment with antihistamines, oral antibiotics, lindane lotion and topical and systemic corticosteroids was ineffective. Both were started on PUVA therapy given twice weekly with an initial dose of 20 mg Oxsoralen ultra. After 15 and 13 treatments, respectively, in both patients the insect-bite reaction and itching resolved completely.

Patient 1 again presented 6 months later with persistent insect-bite reactions after a hunting trip, and following a further course of PUVA given twice weekly and after eight treatments, his cutaneous eruptions and pruritus resolved.

Oral psoralen photochemotherapy has been of use for intractable pruritus associated with the acquired immunodeficiency syndrome,[2] nodular prurigo,[3] atopic eczema,[4] and lichen planus.[5] The mode of action for this improvement is uncertain. The total number of circulating T lymphocytes, as well as T-helper and T-suppressor subsets, are decreased after repeated exposure to PUVA both in normal controls and in patients with psoriasis.[2,6,7] Also, immunological reactions produced by other HLA-DR antigen-expressing cells such as monocytes, Langerhans cells and certain bone marrow precursor cells are inhibited.[8] This leads to diminished delayed hypersensitivity responses.[7,8]

The improvement with PUVA seen in our patients who had persistent insect-bite reactions has not been previously reported, and this therapy should be considered for those patients who are often unresponsive to other treatments.

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current policy is to use the sums at their disposal to encourage young investigators whose proposals might otherwise be viewed unsympathetically by other bodies.

The funds for research awarded annually by the Trustees continue to rise, being £96,000 in 1987, £126,000 in 1988 and £150,000 in 1989. A wide variety of projects are currently being supported by The Skin Disease Research Fund.

Much emphasis is placed by the Trustees on regular progress reports from the successful applicants, and a member of the Grants Advisory Committee is normally appointed to liaise with the grant holder. The invitation to submit applications is by way of a notice in the *British Journal of Dermatology* and the Fund does not usually consider *ad hoc* applications. Readers, and particularly members of the BAD, are asked to attract donations to the Fund, and these and enquiries concerning research applications should be sent to the Honorary Appeal Secretary, Dr K.G.Gowers, JP, ACIB, 12 Knighton Close, South Croydon, Surrey CR2 6DP, telephone no. 081-668-4300.

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The Third Meeting of the Panamerican Society for Pigment Cell Research will take place at the University of Alberta 11–13 July 1991. There will be four keynote speakers, workshops, symposia and plenary and poster sessions. For further information contact: Yolande Matsusaki, Conference Centre, 4 Lister Hall, University of Alberta, Edmonton, Alberta T6G 2H6, Canada and Dr Kowichi Jimbow, Division of Dermatology and Cutaneous Sciences, Faculty of Medicine, 260G Clinical Research Wing, Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta T6G 2S2, Canada.

Dystrophic Epidermolysis Bullosa Research Association

The Dystrophic Epidermolysis Bullosa Research Association (DEBRA) is a national charity working to fund research into epidermolysis bullosa and to support children and adults with the condition. Research on epidermolysis bullosa is in progress at a number of centres and applications are invited for grants for those interested in the study of this condition. Those interested should contact: the Director, DEBRA, 1 Kings Road, Crowthorne, Berkshire RG11 7BG, telephone 0344 771961, from whom a grant application form can be obtained.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
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British Journal of Dermatology, Volume 123, Number 5, November 1990

CONTENTS PAGE

Immunobiological studies on the alopecic (DEBR) rat 557
H.J.MICHIE, C.A.B.JAHODA, R.F.OLIVER AND T.A.POULTON

The presence of autoantibody to recombinant lipocortin-1 in patients with psoriasis and psoriatic arthritis 569
J.K.RIVERS, M.R.PODGORSKI, N.J.GOULDING, E.WONG, J.A.MCGRATH, R.J.FLOWER AND M.W.GREAVES

Radiobiological studies in the naevoid basal cell carcinoma syndrome 573
JULIA A.NEWTON, ANNE KOBZA BLACK, C.F.ARLETT AND JANE COLE

Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin 581
W.P.ARNOLD, CANDIDA A.E.M.VAN HOOIJDONK, P.D.MIER AND P.C.M.VAN DE KERKHOF

The significance of Leu 8 negative T cells in lymphoid skin infiltrates: malignant transformation, selective homing or T-cell activation? 587
J.U.RIJLAARSDAM, D.M.BOORSMA, P.DE HAAN, S.SAMPAT AND R.WILLEMZE

Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol 595
R.J.SAFFORD, D.A.BASKETTER, C.F.ALLENBY AND B.F.J.GOODWIN

Cutaneous sodium lauryl sulphate irritation potential: age and regional variability 607
ANASTASIA B.CUA, K.-P.WILHELM AND H.I.MAIBACH

Conjunctival involvement in pemphigus vulgaris: a clinical, histopathological and immunofluorescence study 615
EMMILIA HODAK, I.KREMER, M.DAVID, B.HAZAZ, A.ROTHEM, P.FEUERMAN AND MIRIAM SANDBANK

Effect of long-term PUVA treatment of psoriasis on the collagen and elastin gene expression and growth of skin fibroblasts *in vitro* 621
A.OIKARINEN, L.ALA-KOKKO, M.TAMMINEN, J.KARVONEN, T.REUNALA, M.KALLIOINEN AND M.HANNUKSELA

Enhanced percutaneous absorption of a novel topical cyclosporin A formulation and assessment of its immunosuppressive activity 631
J.I.DUNCAN, S.N.L.PAYNE, A.J.WINFIELD, A.D.ORMEROD AND A.W.THOMSON

Cyclosporin A in combination with photochemotherapy (PUVA) in the treatment of psoriasis 641
P.PETZELBAUER, H.HÖNIGSMANN, K.LANGER, BARBARA ANEGG, P.STROHAL, A.TANEW AND K.WOLFF

A case of T-cell lymphoma associated with panniculitis, progressive pancytopenia and hyperbilirubinaemia 649
K.TANAKA, Y.HAGARI, Y.SANO, S.SHIMAO AND K.NANBA

Treatment of severe acne with isotretinoin in patients with inflammatory bowel disease 653
K.M.GODFREY AND M.P.JAMES

Multiple glomus tumours: successful treatment with electron beam irradiation 657
K.NISHIMOTO, M.NISHIMOTO, S.YAMAMOTO, T.NAKAGAWA, T.TAKAIWA AND M.TANABE

Pachyonychia congenita complicated by hidradenitis suppurativa: a family study 663
P.TODD, J.GARIOCH, M.RADEMAKER, W.SUSSKIND, C.GEMELL AND J.THOMSON

Profound digital collagen atrophy: a new cutaneous presentation of adrenal-dependent Cushing's syndrome 667
R.W.GROVES, L.M.MACDONALD AND D.M.MACDONALD

Society Proceedings

Proceedings of the 248th Meeting of the Netherlands Society for Dermatology and Venereology 673

Correspondence

Acantholytic lesions in the vagina 691
F.BRANDRUP, J.PETRI AND J.AEGIDIUS

Pityriasis rubra pilaris in Indians 692
A.J.KANWAR, SURRINDER KAUR

Direct antiproliferative effect of cyclosporin A on cultured keratinocytes and fibroblasts 693
B.J.NICKOLOFF AND R.S.MITRA

Persistent bite reactions responsive to photochemotherapy 693
B.E.BEACHAM AND D.KURGANSKY

Book Review 695

News and Notices 696

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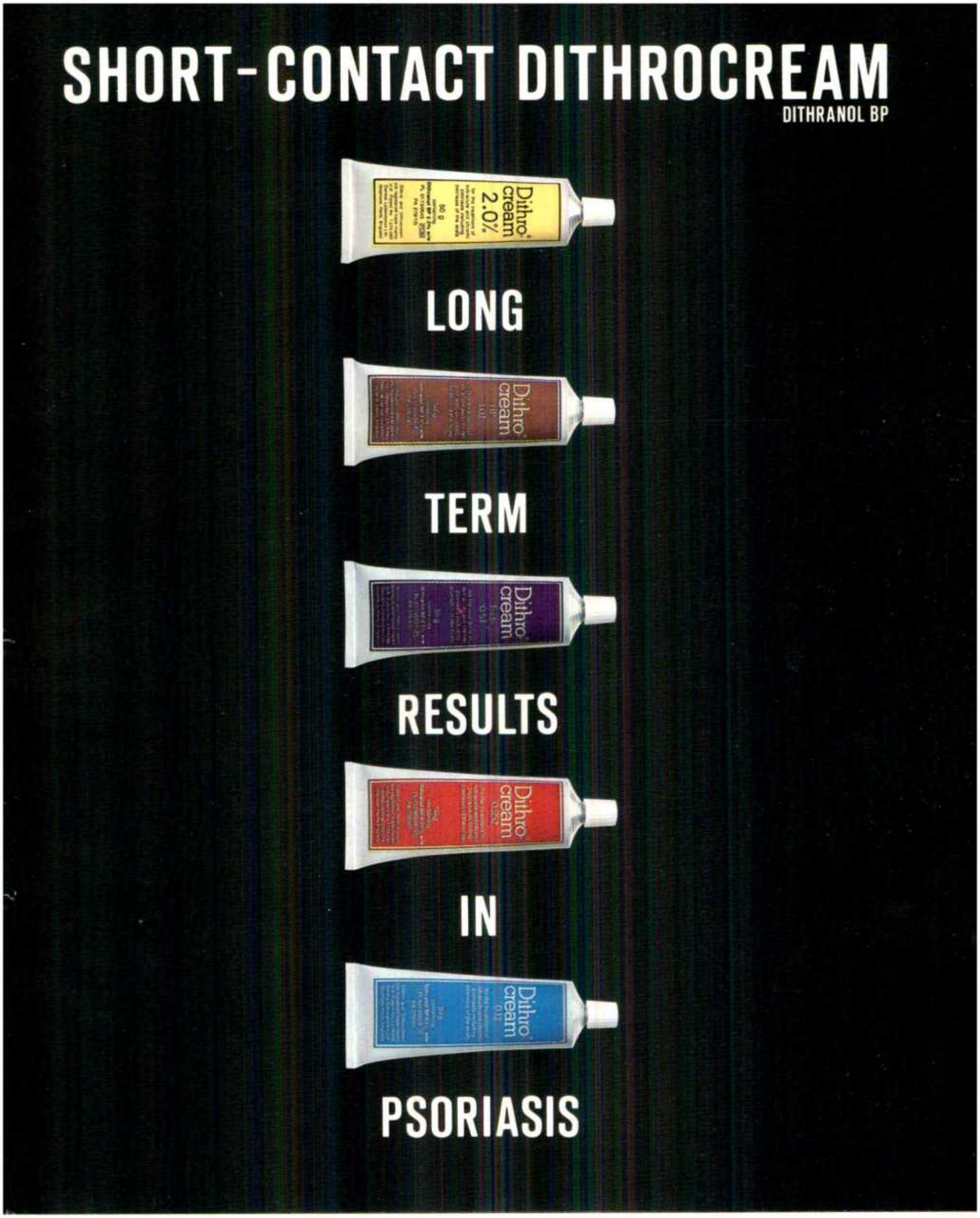

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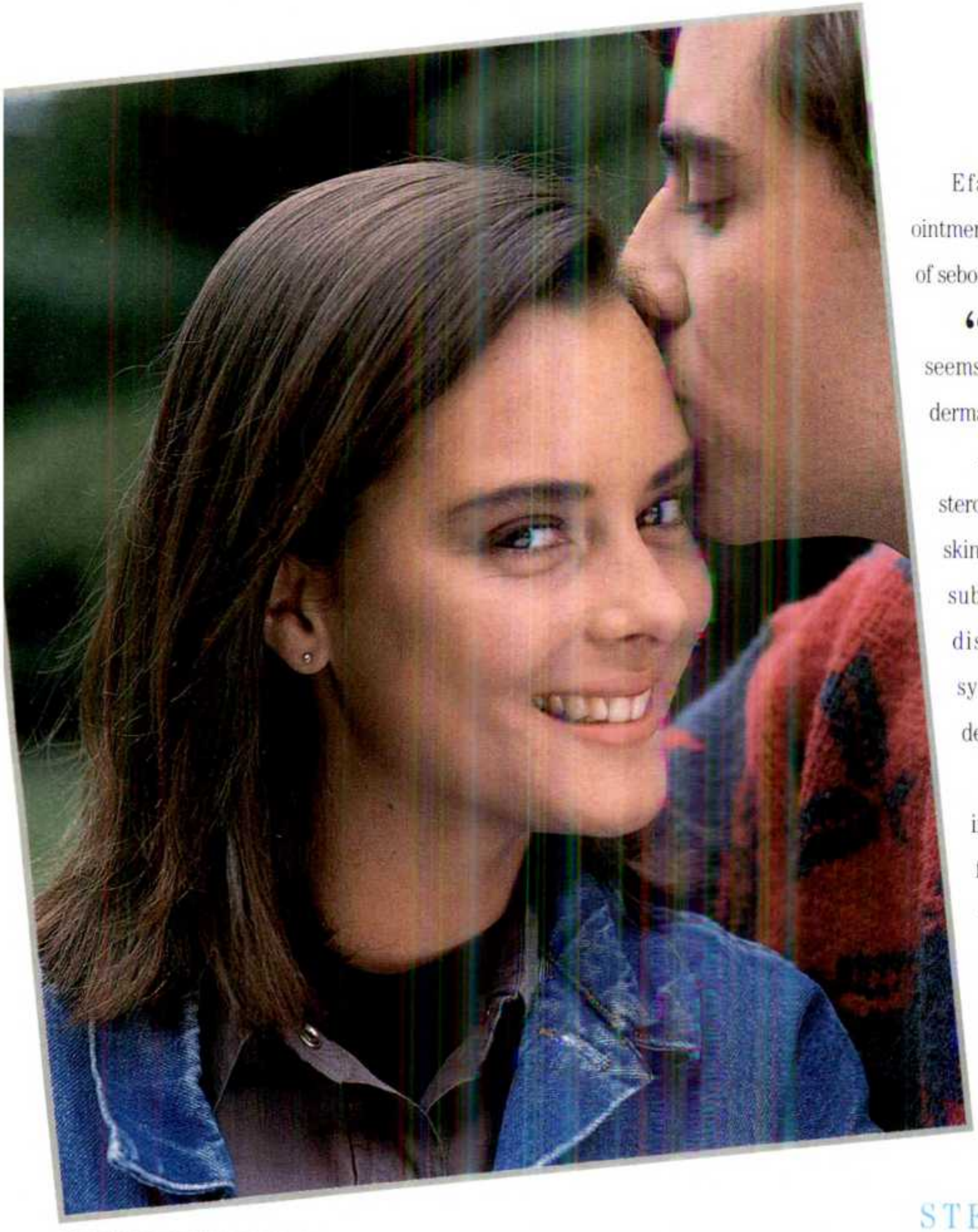

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2. Data on File, Scotia Pharmaceuticals Ltd.

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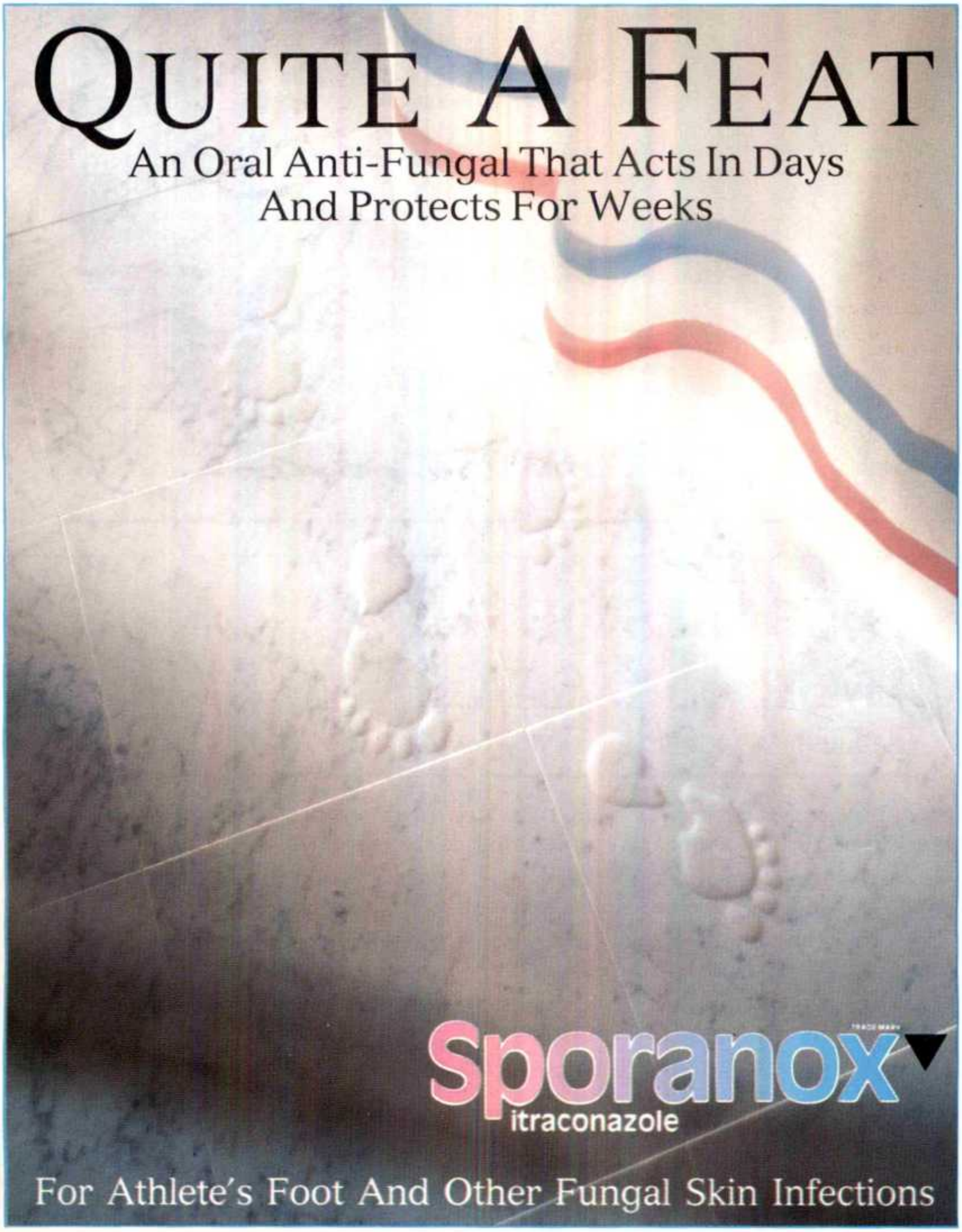
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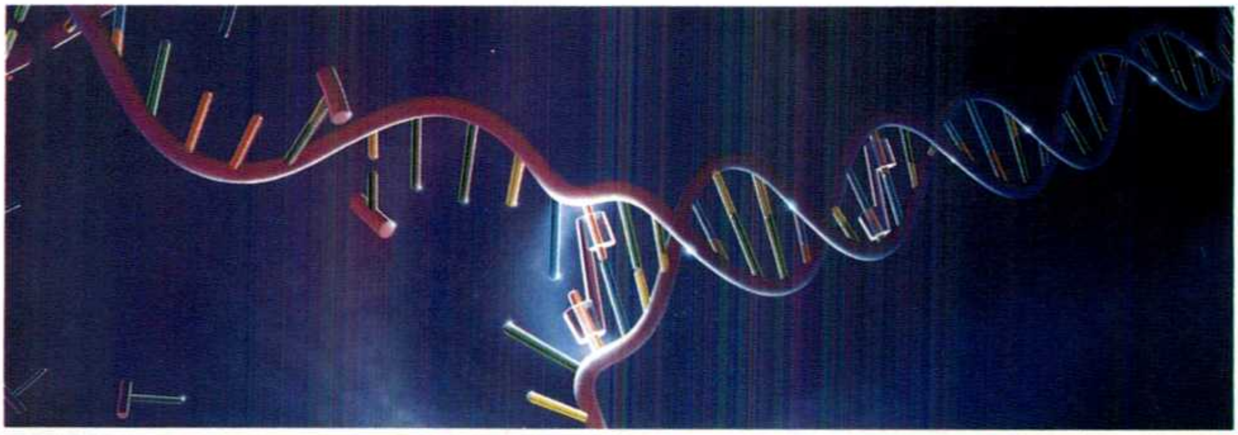

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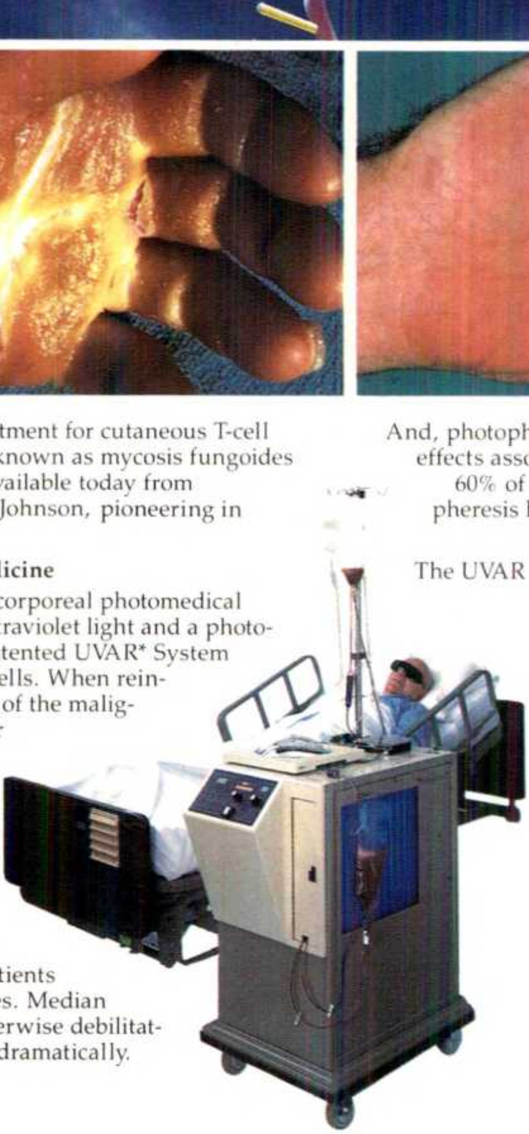

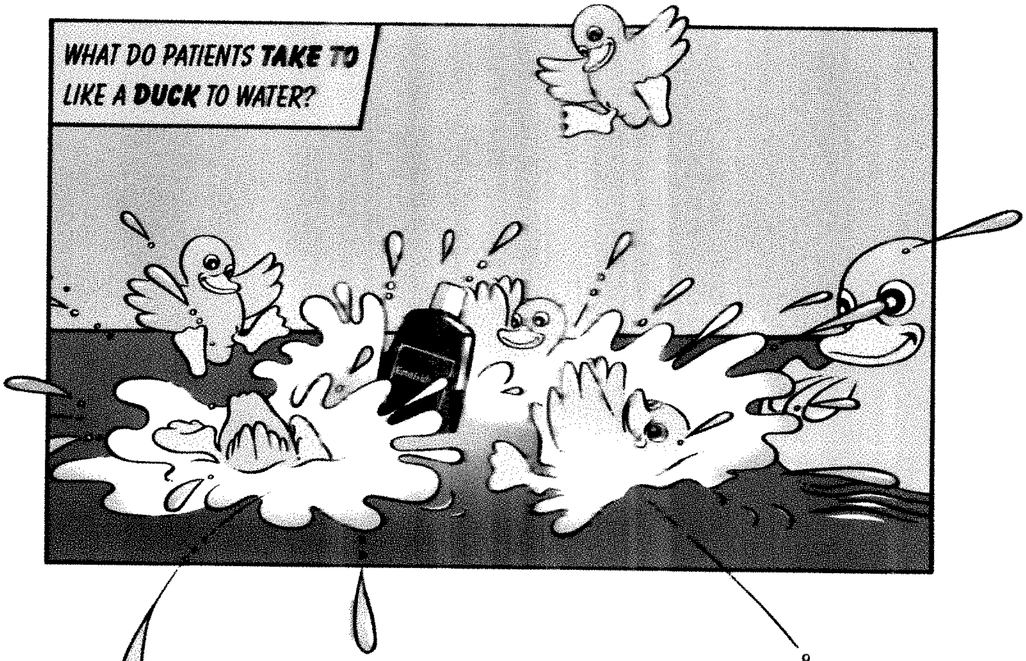
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British Journal of Dermatology (1990) **123**, 699–705.

Langerhans cells but not monocytes are capable of antigen presentation *in vitro* in corticosteroid contact hypersensitivity

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SUMMARY

Corticosteroids suppress delayed-type hypersensitivity (DTH) reactions *in vivo* and impair lymphoid cell functions *in vitro*. In contact hypersensitivity (CHS) to corticosteroids, however, the corticosteroids are capable of inducing DTH responses *in vivo*. The present study examined the capacity of corticosteroids to induce *in vitro* proliferation of T lymphocytes from patients with CHS to corticosteroids. With peripheral blood mononuclear adherent cells as antigen-presenting cells (APC) and hydrocortisone-17-butyrate (H-17-B) as hapten, no proliferation responses were detected of T lymphocytes from patients with CHS to H-17-B. However, when epidermal Langerhans cells (LC) were used as APC, weak proliferation responses were observed.

Epidermal Langerhans cells (LC) are dendritic antigen-presenting cells (APC) and they participate in the induction and elicitation of delayed-type hypersensitivity (DTH) reactions.[1] Corticosteroids suppress DTH responses by impairing the function of APC[2-6] and by inhibiting proliferation of peripheral blood lymphocytes.[7,8] In patients with contact hypersensitivity (CHS) to corticosteroids, however, the corticosteroids themselves are capable of inducing inflammatory reactions, resulting in the clinical picture of allergic contact dermatitis.[9,10] The diagnosis of CHS to corticosteroids can be difficult, probably because the anti-inflammatory action of corticosteroids counteracts the epicutaneous test reaction.[9,11]

In the present *in vitro* study, we investigated the cellular interactions between APC and T lymphocytes in CHS to corticosteroids. Our aim was also to examine whether an *in vitro* test would be useful in assessing CHS to different topical corticosteroids.

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METHODS

Patients and control subjects

Eight patients with CHS to one or several topical corticosteroids were studied. Epicutaneous testing for CHS was performed as described elsewhere.[9] Six healthy subjects without CHS to corticosteroids were the controls.

Separation of epidermal cells

Suction blisters were raised on the clinically healthy abdominal skin of six patients as described previously.[12] The epidermal sheets were treated with 0·25% trypsin (Gibco Ltd, Paisley, Scotland, U.K.) for 30 min at 37°C and then with 0·01% DNase I (Sigma Chemical Co., St Louis, MO, U.S.A.) for 5 min at room temperature, after which the cell suspensions were filtered through a nylon mesh. The epidermal cell suspensions contained on average 2·3% (range 1·3–4·4%) CD1a$^+$ LC as assessed by immunostaining with OKT6 (Ortho Diagnostic Systems Inc., Raritan, NJ, U.S.A.). To study in more detail the APC function of LC the method of Hanau *et al.*[13] with modifications was used to enrich LC from crude epidermal cell suspensions. Immunomagnetic beads precoated with sheep anti-mouse IgG (Dynal A.S., Oslo, Norway) were treated with OKT6 for 2 h at 4°C with a final dilution of antiserum of 1:10 in RPMI 1640 containing 0·1% bovine serum albumin. Washed OKT6-coated immunomagnetic beads were added to epidermal cell suspensions at approximately 5–10 beads to one LC. The cells were centrifuged at 250 ***g*** for 5 min and incubated at 4°C for 45 min with occasional shaking, followed by isolation of rosette-forming LC from non-rosette-forming epidermal cells with a magnet. The rosettes were suspended in cold RPMI 1640 and the magnetic isolation step was repeated three times. The recovered LC-enriched fractions contained between 75 and 87% CD1a$^+$ LC. To obtain LC-depleted fractions for some experiments, after separation of LC-enriched fraction as described above, immunomagnetic beads (bead/cell ratio 1:1) were added to the epidermal cell suspension and the rosetting procedure was repeated once. No CD1a$^+$ cells were detected in the LC-depleted epidermal cell fractions ('keratinocytes').

Separation of peripheral blood mononuclear cells

Peripheral blood mononuclear leucocytes (PBML) were obtained by Ficoll–Isopaque centrifugation of venous blood. The PBML contained on the average 84% lymphocytes and 16% monocytes as assessed by non-specific esterase staining.[14] Monocytes were purified on the basis of their adherence to plastic surfaces and T lymphocytes by rosette formation with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes as described elsewhere.[15] The mononuclear adherent cells ('AC') contained over 90% cells that stained for non-specific esterase which is characteristic for monocytes. T-lymphocyte-enriched fractions ('T lymphocytes') contained over 90% CD3$^+$ T cells as assessed by immunostaining with OKT3 (Ortho Diagnostic Systems Inc., Raritan, N.J., U.S.A.), and less than 0·1% monocytes according to the non-specific esterase staining.

Cell cultures

The PBML were cultured as such or, following further separation of T lymphocytes and mononuclear adherent cells (AC), T lymphocyte cultures supplemented with APC (LC or AC) were set up. In some experiments T cells were cultured with crude epidermal cells or keratinocytes. Unless otherwise specified, 25 × 10^3 cells per well were cultured on V-bottomed microtitre plates. The cells were cultured in RPMI 1640 supplemented with 10% autologous plasma for 6 days unless otherwise stated. The cultures were stimulated with 5 μg/ml purified protein derivative of tuberculin (PPD; Statens Seruminstitut, Copenhagen, Denmark) and/or hydrocortisone-17-butyrate (H-17-B; Gist-Brocades, Delft, The Netherlands, supplied through Algol Oy, Helsinki) or hydrocortisone-free alcohol (Oriola Oy, Espoo, Finland) at various concentrations. The cultures were pulsed with 0·125 μCi/well of ^{125}I-labelled iododeoxyuridine for the final 18 h of incubation and the isotope incorporation measured with a gamma counter.[16] The results were expressed as stimulation indices (SI = uptake of isotope in stimulated culture/uptake of isotope in non-stimulated control culture). SI values of at least 2·5 were regarded as positive. The c.p.m. values of control cultures were as a rule less than 100.

RESULTS

Effect of corticosteroids on lymphocyte proliferation

In preliminary experiments PBML from five patients with CHS to H-17-B were cultured in the presence of H-17-B at various concentrations (0·1–10 μg/ml). In contrast to the usual increase in proliferation seen in CHS to other haptens, an inhibition was observed even at low concentrations. After 6 days of culture with 1·0 μg/ml of H-17-B, for example, the measured DNA synthesis was 66·4% (range 49–84%) of that of untreated controls.

For further investigation of the antiproliferative effect of corticosteroids, PBML from healthy control subjects were stimulated with 5 μg/ml purified protein derivative of tuberculin (PPD) in the presence of H-17-B or hydrocortisone at various concentrations. Table 1 shows that there was a dose-dependent decrease in DNA synthesis in the presence of these corticosteroids.

Capacity of enriched LC to present corticosteroids to T lymphocytes

Enriched LC were recovered as LC immunomagnetic bead rosettes; these were so stable that the beads could not be released. Since the immunomagnetic beads might have impaired LC

TABLE 1. Effect of corticosteroids on PPD-induced proliferation of peripheral blood mononuclear leucocytes of normal subjects

	H-17-B (μg/ml)			Hydrocortisone (μg/ml)		
Subject	0·1	1	10	0·1	1	10
1	74*	32	15	71	81	50
2	79	75	36	54	47	19
3	93	58	40	56	29	15
4	33	34	28	70	45	14
5	82	74	60	105	50	27
6	31	13	13	66	51	48
Mean	65·3	47·7	32·0	70·3	50·5	28·8

The cells were stimulated with 5 μg/ml of PPD and cultured for 6 days.

* Percentage of PPD-induced proliferation = (c.p.m. in culture with PPD supplemented with corticosteroid/c.p.m. in culture containing PPD) × 100%.

function, the antigen-presenting capacity of LC rosettes was compared to AC and LC in epidermal cell suspensions; 25 × 10³ T lymphocytes were co-cultured with approximately 1250 LC or AC, or 25 × 10³ crude epidermal cells. The results of two experiments with 5 μg/ml PPD showed that the LC rosettes retained an antigen-presenting capacity, which, however, did not seem as good as that of crude epidermal cells. Nevertheless, the APC function of enriched LC was better than that of AC (Table 2).

The LC-enriched fractions and crude epidermal cells, in contrast to AC and keratinocytes, were able to present H-17-B to T lymphocytes, resulting in proliferation responses, as shown in Table 3. The optimal concentration of H-17-B in these experiments was low, i.e. 0·2–0·8 μg/ml.

TABLE 2. Comparison of epidermal cells, enriched Langerhans cells and peripheral blood mononuclear adherent cells as antigen-presenting cells in PPD-induced T lymphocyte proliferation

	Proliferation (SI)		
Patient	Epidermal cells	Enriched Langerhans cells	Peripheral blood adherent cells
2	35·8	26·0	17·9
6	96·1	56·2	37·8

T lymphocytes (25 × 10³) were supplemented with approximately 1250 enriched Langerhans cells or peripheral blood mononuclear adherent cells, or 25 × 10³ crude epidermal cells. The cells were cultured in the presence of 5 μg/ml PPD for 6 days.

SI = c.p.m. in stimulated culture/c.p.m. in non-stimulated control culture. The c.p.m. value of control cultures was, as a rule, less than 100.

TABLE 3. Hydrocortisone-17-butyrate-induced T-lymphocyte proliferation and epicutaneous test results in patients with contact hypersensitivity to hydrocortisone-17-butyrate

	Proliferation (SI) in T-cell cultures supplemented with:				
Patient	Langerhans cells	Adherent cells	Epidermal cells	Keratinocytes	Epicutaneous tests*
1	13·6	1·3	—	—	+ + +
2	5·2	2·2	—	—	+
3	4·6	1·5	—	—	+ +
4	2·6	1·0	—	—	+
5	4·5	0·4	3·1	0·9	+
6	3·9	1·1	2·8	1·3	+ +

T lymphocytes (25 × 10³) were supplemented with 1250 Langerhans cells or peripheral blood adherent cells or 25 × 10³ epidermal cells or keratinocytes. The results were obtained at the optimal concentration of hydrocortisone-17-butyrate (0·2–0·8 μg/ml) and optimal culture time (5–6 days).

* Tests performed[9] as described elsewhere.

TABLE 4. Kinetics of the proliferation responses to hydrocortisone-17-butyrate

	Culture time (days)				
Patient	4	5	6	7	8
1	6·2*	9·7	10·1	ND	ND
2	3·1	5·2	4·0	3·3	2·7
5	2·9	4·4	4·5	4·2	3·1

T lymphocytes (25×10^3) were supplemented with 1250 Langerhans cells and cultured in the presence of 0·8 μg/ml hydrocortisone-17-butyrate.

ND, not done.

* Stimulation index (SI).

TABLE 5. Proliferation responses and epicutaneous test results to hydrocortisone-17-butyrate and hydrocortisone

	Proliferation (SI)						Epicutaneous test*	
	H-17-B (μg/ml)			Hydrocortisone (μg/ml)			H-17-B	Hydrocortisone
Patient	0·2	0·8	3·1	0·2	0·8	3·1		
1	13·6	9·7	7·1	1·7	0·6	0·8	+ + +	−
6	3·9	3·8	2·5	7·2	6·3	3·6	+ +	+

T lymphocytes (25×10^3) were supplemented with 1250 Langerhans cells and cultured for 5 days in the presence of H-17-B or hydrocortisone.

* Tests performed[9] as described elsewhere.

To study the kinetics of the reaction, the cells were harvested at various time-points; the optimal culture time was 5–6 days (Table 4).

The results of two experiments performed with H-17-B and hydrocortisone are shown in Table 5. The patient with a positive epicutaneous test result to H-17-B and hydrocortisone showed proliferative responses to both corticosteroids, whereas the second patient who had a positive epicutaneous test only to H-17-B did not respond to hydrocortisone *in vitro*.

DISCUSSION

The present study suggests that in contrast to peripheral blood mononuclear adherent cells, epidermal Langerhans cells are capable of presenting corticosteroid haptens to T lymphocytes from patients with contact hypersensitivity to corticosteroids. The LC were enriched with a simple and rapid immunomagnetic bead technique, modified from Hanau *et al.*[13] The LC fractions obtained were relatively pure, i.e. contained 75–87% LC. However, with PPD as the antigen, the antigen-presenting capacity of these fractions seemed somewhat impaired when compared to that of crude epidermal cell suspensions with corresponding quantities of LC. This impairment could be explained by steric hindrance created by the magnetic beads still bound to LC via the CD1a epitopes. On the other hand, with H-17-B the proliferation responses with LC-enriched fractions were similar to those with crude epidermal cells.

The lack of antigen-presenting capacity of peripheral blood AC observed with corticosteroids could have several explanations. To act as an antigen the particular corticosteroid hapten has to bind to a carrier protein: during the sensitization this binding could take place in the epidermis. Thus, the elicitation reactions could be specific for this hapten–carrier complex and the putative carrier protein could be present in the epidermis but not in the peripheral blood. Such epidermal carrier proteins would be transferred to the *in vitro* proliferation assays with enriched epidermal LC fractions. Another possibility is that the carrier protein is a structural part of the LC cell membrane and that peripheral blood AC could be devoid of this structure, or alternatively it could be hidden.

It has been shown that in some patients with CHS to nickel, the *in vitro* proliferative response of T lymphocytes to nickel can be induced with LC but not AC as APC,[17] a phenomenon similar to the one we observed in the present study with corticosteroids. In those patients in whom the *in vitro* response to nickel can be induced with AC as APC, the antigen-presenting capacity of LC exceeds that of AC.[17,18] Furthermore, it has been shown that in CHS to nickel more than half of the nickel-specific T lymphocytes cloned from allergic contact dermatitis lesions are able to proliferate with LC but not with AC as APC.[19] The corticosteroid-specific T lymphocytes could thus have characteristics similar to these nickel-specific clones. One more explanation is provided in a recent study which shows that freshly isolated LC are very efficient presenters of soluble antigen *in vitro*.[20]

Several *in vitro* studies have previously shown that hydrocortisone and all other corticosteroids studied inhibit the antigen-presenting capacity of both LC and AC.[2–5] Even short-term incubation of LC or AC with corticosteroids prior to adding them to responder T lymphocytes is enough to impair their APC function.[3] Although such data do not exist for H-17-B, it seems likely that the contrasting results observed for AC and LC cannot be explained by differential actions of corticosteroids to AC and LC.

The optimal concentrations of corticosteroids in cell cultures were 0·2–0·8 μg/ml, concentrations which were lower than the optimal concentrations with most haptens, e.g. 1·25–6·25 μg/ml for nickel;[21] the responses observed were also weak. The weak proliferative responses and the low optimal concentrations of corticosteroids in these experiments could be due to the pharmacological properties of corticosteroids. The higher amount of antigen available in concentrations over 1 μg/ml could well be counteracted by the increased anti-inflammatory[3,5] and antiproliferative[7] action of corticosteroids in these *in vitro* experiments.

Because of the difficulty in interpreting epicutaneous test results in corticosteroid contact hypersensitivity, an *in vitro* test is desirable. Since the positive lymphocyte transformation test described requires epidermal cells or LC in addition to T lymphocytes obtained from venous blood, the use of this *in vitro* approach would be limited to those cases with widespread dermatoses that would not permit epicutaneous testing.

ACKNOWLEDGMENTS

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The cytokeratin expression of cultured human foetal keratinocytes

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SUMMARY

Skin samples taken from foetuses of 15–19 weeks gestational age and keratinocytes were cultured by the 3T3 feeder method or in serum-free MCDB 153 medium on 16 mm coverslips. Keratinocytes taken from paediatric circumcisions and patients undergoing plastic surgery were also cultured using the 3T3 feeder method. A panel of monoclonal antibodies against a number of cytokeratins and differentiation markers were used in the PAP technique to analyse the cells. Cryostat sections taken from the donor skin samples were stained simultaneously. Foetal skin expressed the cytokeratins 7, 13 and 19 that were not observed postnatally. This cytokeratin expression was maintained in both culture conditions and also observed in paediatric and adult keratinocytes. Cytokeratin 7 was expressed on a greater proportion of foetal cells than *in vivo*, whereas the expression of 13 and 19 decreased. All keratinocytes expressed vimentin, transferrin receptor and Ki67 (proliferating cell antigen), while a small proportion expressed involucrin throughout culture, indicating their high level of differentiation and proliferation. No differences were observed between low and high density cultures. Foetal keratinocytes cultured in MCDB 153 did not reach confluence and stronger staining of differentiation markers was observed in these cells. These results show that the *in vivo* differences in cytokeratin expression of foetal and adult keratinocytes disappear in culture.

The cytokeratins are a complex group of 20 polypeptides that form intermediate filaments in various human epithelial cells.[1] The cytokeratin profile of keratinocytes is closely related to differentiation and may be used to evaluate the clonal origin and state of the cell. Cytokeratins 1 and 10 are located in the suprabasal layers and are characteristic of keratinizing tissues, while the proliferating cells of the basal layer express numbers 5 and 14 that are characteristic of all stratified epithelia.[2] The cytokeratin pattern may show cellular heterogeneity in different locations, such as the foot sole epidermis,[3] and can be modulated by environmental conditions and disease.[4] Cytokeratin expression can also be experimentally manipulated by changing cell culture conditions, for example cytokeratin 1 is induced by vitamin A depletion[5] or air exposure[6] while differentiation is retarded in cells cultured in low calcium media.[7]

During embryonic and foetal development the epidermis changes from a single cell layer covered by the periderm to a more complex stratified epithelium and finally to a keratinized epidermis.[8] These changes are reflected by a changing cytokeratin pattern, so that differences between foetal and adult skin have been observed.[7] The aim of this study was to extend these observations and determine the cytokeratin expression of foetal skin throughout culture in comparison to that of adult and paediatric cultured keratinocytes.

METHODS

Tissue

Fresh foetal skin was obtained from prostaglandin-induced terminations of 14–19 weeks estimated gestational age (EGA). Paediatric skin was obtained from patients (2–10 years) undergoing circumcision and adult skin from patients undergoing plastic surgery.

Keratinocyte culture

Keratinocytes were cultured using the 3T3 feeder method of Rheinwald and Green.[9] Due to the difficulty of foetal culture, cells were also cultured in MCDB 153 medium supplemented with bovine pituitary extract (BPE).[10] After trypsin treatment, 2×10^5 (foetal) or 5×10^4 cells were plated on to 16 mm glass coverslips in 24-well plates and incubated at 37°C in a humid 95% air/5% CO_2 environment. The medium was changed every third day for both culture methods. At various intervals the cells were washed with phosphate-buffered saline, the coverslips lifted out and fixed for 20 min in acetone for immunohistochemical analysis.

Immunohistochemistry

Biopsies were snap frozen and 5–7 μm cryostat sections of foetal and adult skin were mounted on the same gelatin-coated slide, acetone fixed for 20 min and simultaneously immunostained with cultured cells on coverslips by the unlabelled peroxidase–antiperoxidase (PAP) method. Cultured cells were examined at primary and secondary culture, at the start of colony formation and at confluence. The sources, specificities and dilutions of the primary layer monoclonal antibodies (MAbs) are shown in Table 1. MAbs were applied to air-dried sections and coverslips for 60 min at room temperature. After washing these were incubated with rabbit anti-mouse Ig (Dakopatts 1:20) in 20% normal human AB serum for 30 min, followed by a 30 min incubation with 1/100 dilution of PAP complex (Dakopatts, High Wycombe, U.K.). The bound peroxidase was visualized using the diaminobenzidine/H_2O_2 reaction. Sections and coverslips were counterstained in Harris' haematoxylin and dehydrated. Slides were mounted in DPX and coverslips were inverted onto slides with DPX. Controls included the replacement of primary antibody with TBS or normal mouse immunoglobulin. The intensity of staining was assessed and graded on a scale from 0 to + +. Control slides and coverslips were uniformly negative.

RESULTS

Foetal cell culture

Two methods of cell culture were used to maximize foetal keratinocyte yield and growth. The 3T3 feeder method gave rise to confluent keratinocyte sheets after a period of 4–5 weeks, whereas the MCDB 153 medium culture resulted in colony formation but without confluence

TABLE 1. Monoclonal antibody panel

Antibody	Specificity	Source	Dilution
RPN 1160	Cytokeratin 18	Amersham*	1/20
RPN 1162	Cytokeratin 7	Amersham	1/20
RPN 1164	Type II cytokeratins	Amersham	1/20
RPN 1165	Cytokeratin 19	Amersham	1/20
RPN 1166	Cytokeratin 8	Amersham	1/20
K 8.12	Cytokeratin 13,16	Sigma†	1/100
K 8.13	Cytokeratin Range	Sigma	1/100
K 8.60	Cytokeratin 10,11	Sigma	1/100
Ks 13.1	Cytokeratin 13	Cymbus Bioscience‡	1/10
GB 5	Strata granulosum spinosum + germinatum	Seralab§	1/50
GB 6	Basal keratinocytes	Seralab	1/50
Ber. T9	Transferrin receptor	Dakopatts¶	1/10
Ki 67	Proliferating cell	Dakopatts	1/10
EGF-r	Epidermal growth factor receptor	Amersham	1/50
DK 22	HLA-DR	Amersham	1/20
Vim 13.2	Vimentin	Serotec**	1/20
W6/32	HLA class I	Serotec	1/20
Involucrin (polyclonal)	Involucrin	Dr F. Watt	1/250

* Amersham, Little Chalfont, U.K.
† Sigma Chemical Co., Poole, Dorset, U.K.
‡ Cymbus Bioscience, Southampton, U.K.
§ Sera-Lab Ltd, Crawley Down, Sussex U.K.
¶ Dakopatts, High Wycombe, Bucks, U.K.
** Serotec, Kidlington, Oxford, U.K.

(Fig. 1). A higher seeding density was required for the foetal rather than for the paediatric or adult keratinocytes. Differences in cytokeratin expression were not observed between the two cell culture methods, possibly as a result of the addition of BPE to MCDB 153 resulting in normal Ca^{2+} concentration.[7] Stronger staining patterns for differentiation and proliferation markers were observed on small keratinocyte cultures grown in MCDB 153 medium.

Cytokeratin expression

The differences in cytokeratin expression observed in adult and foetal keratinocytes *in vivo* and in 3T3 feeder cell culture are shown in Table 2. Cells cultured from paediatric foreskin were similar to adult keratinocytes throughout culture, so that no distinction has been made. K 8.13, an antibody against a range of cytokeratins, gave good positive staining in all skin and cultured cell samples studied. The antibody RPN1164 (against type II cytokeratins) faintly stained the suprabasal layers of foetal skin over 15 weeks EGA but was negative in adult skin. However, in culture all cells strongly expressed the antigens recognized by this antibody (Fig. 2).

Cytokeratin 7 was weakly expressed in the suprabasal layers of foetal skin between 13–16 weeks EGA but was not observed in the adult. In culture a small proportion of both adult and foetal keratinocytes expressed this cytokeratin (Fig. 3). Foetal skin stained strongly for cytokeratin 13 and this expression was maintained *in vitro* where adult keratinocytes were also

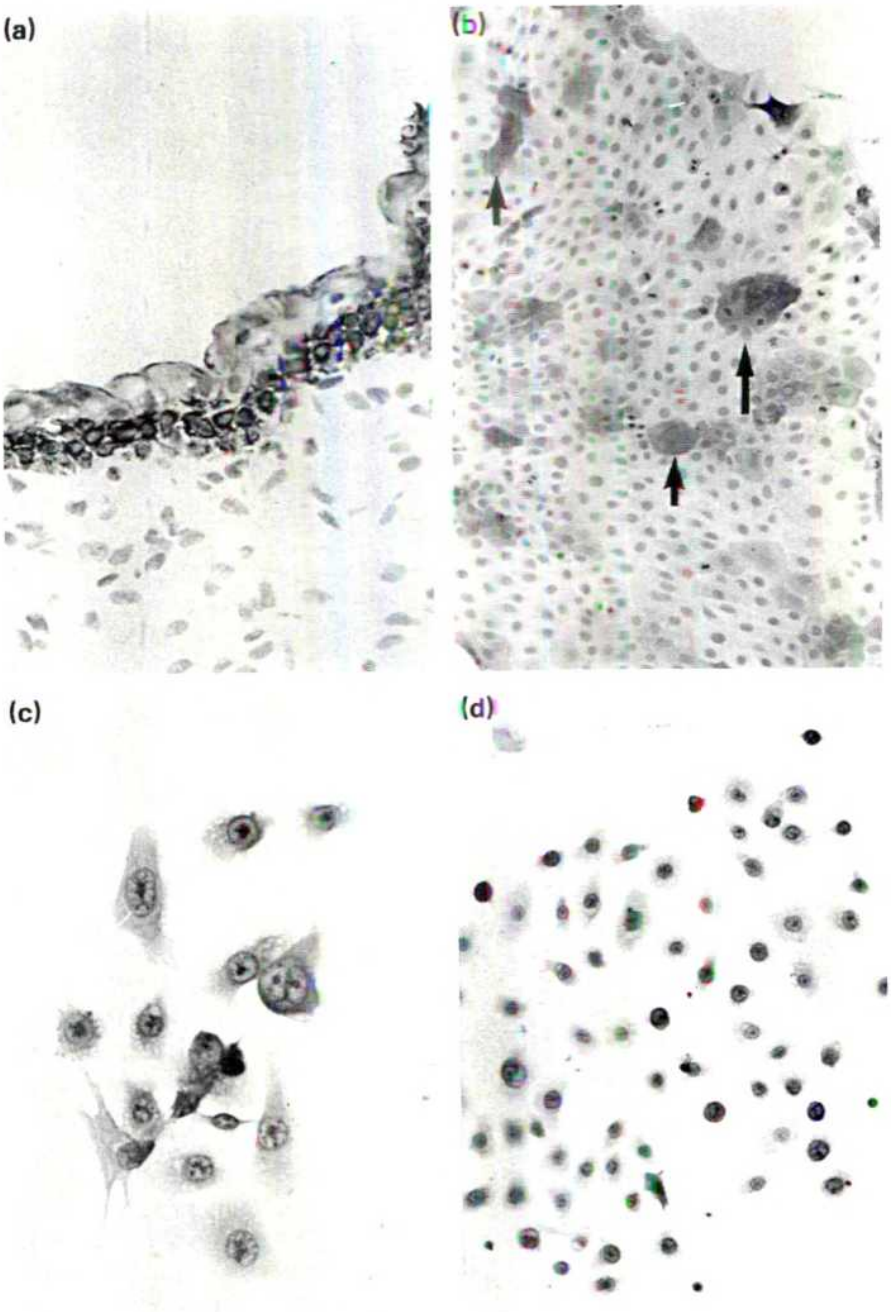

FIGURE 1. (a) Foetal skin at 16 weeks EGA stained with RPN 1165 against cytokeratin 19, showing positive basal cells, × 400. (b) Foetal keratinocytes grown on 3T3 feeder cells at 4 weeks showing cytokeratin 19 positive cells, × 200. (c) and (d) Foetal cells grown in MCDB 153 medium after 3 weeks showing: (c) Ki67 proliferating cell positive cells, × 400 and (d) vimentin positive cells, × 200. Note the smaller colonies and separated cells in MCDB 153 cultured cells, compared to 3T3 feeder cultured keratinocytes.

TABLE 2. The cytokeratin expression of foetal and adult keratinocytes

Cytokeratin	Foetal skin	Culture	Adult skin	Culture
Type II (1164)	+ (supra)	+ + +	–	+ + +
7 (1162)	+ (supra)	+ +	–	+ +
13, 16 (8, 12)	+ + +	+ +	+ (patchy basal)	+ +
13 (Kr.13.1)	+ + +	+ +	–	+ +
18 (1160)	+ (supra basal)	+ +	–	+ +
19 (1165)	+ + (basal)	+	–	+

—, Negative staining.
+, Occasional positive cells present.
+ +, Positive cells consistently present.
+ + +, Abundant positive cells present.

positive. Patchy staining in adult basal cells was observed with the antibody Ks 8.12 that may be due to cross-reactivity with cytokeratin 15.[4] However, strong staining occurred on foetal skin and all cultured keratinocytes with this antibody, indicating the presence of cytokeratins 13 and 16. Cytokeratin 19 was absent in adult skin but was strongly expressed in the foetal basal cells (Fig. 1). A small proportion of cultured cells from both adult and foetal skin also expressed this cytokeratin. Cytokeratin 18 was not expressed on adult skin, unlike foetal skin, but in culture expression of this cytokeratin was observed in adult and foetal keratinocytes. Cytokeratin 8 was absent in all samples while no differences were observed in the expression of cytokeratins 10 and 11.

Differentiation and proliferation markers

Foetal and adult keratinocytes were also compared for the expression of various antigens involved in cell differentiation and proliferation (Table 3). Vimentin, a marker for cells of mesenchymal origin was negative in the epidermis, but in culture a number of keratinocytes were positively stained. A similar reaction pattern was observed for transferrin receptor and Ki67, both markers for proliferation. Involucrin, a marker of keratinocyte terminal differentiation, was present in foetal and adult keratinocytes in culture, but the epidermal staining pattern was different in the adult (granular layer) and the foetus (spinous layer). Epidermal growth factor receptor was increased in foetal cells in culture. HLA class I antigen expression was higher in adult than foetal keratinocytes. Class II antigen-positive Langerhans cells were observed in small numbers in foetal skin and in larger numbers in adult epidermis, but did not survive under the culture conditions used.

DISCUSSION

This study has demonstrated that cultured foetal and adult keratinocytes have a particular cytokeratin pattern that differs from that observed *in vivo*. Unexpected cytokeratin patterns that bear no relationship to the original tissue have previously been observed in certain human cell lines.[1] These results show that although adult keratinocytes in culture express the extra foetal cytokeratins observed *in vivo*, cultured foetal keratinocytes also express cytokeratins not

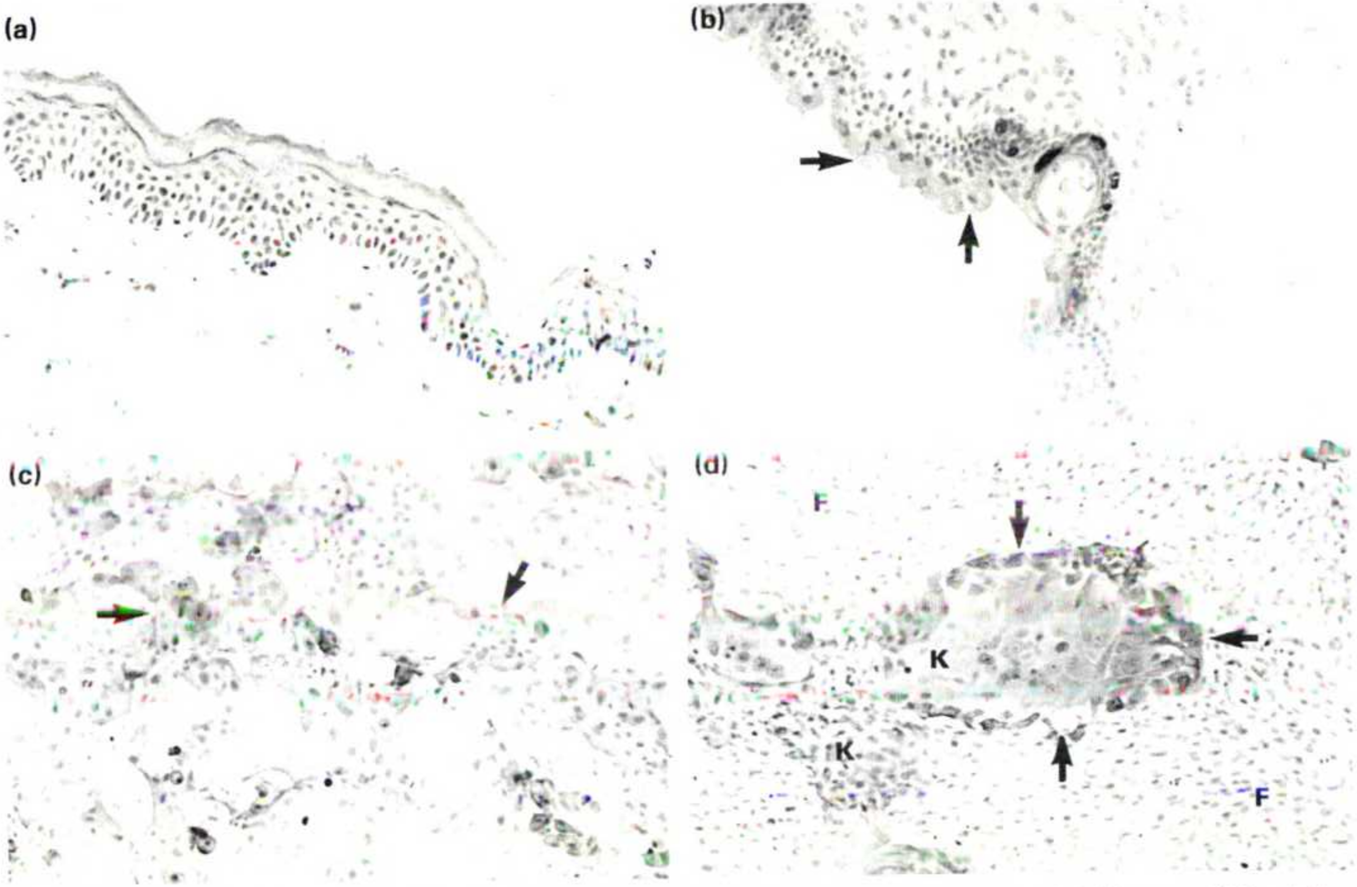

FIGURE 2. Staining pattern observed with the monoclonal antibody RPN1164 against type II cytokeratins on: (a) adult skin, × 200; (b) foetal skin 17 weeks EGA, × 200; (c) adult keratinocytes after 3 weeks in culture, × 200; (d) foetal keratinocytes after 3 weeks in culture, showing negative 3T3 fibroblasts (F) surrounding a positive keratinocyte colony (K), × 100.

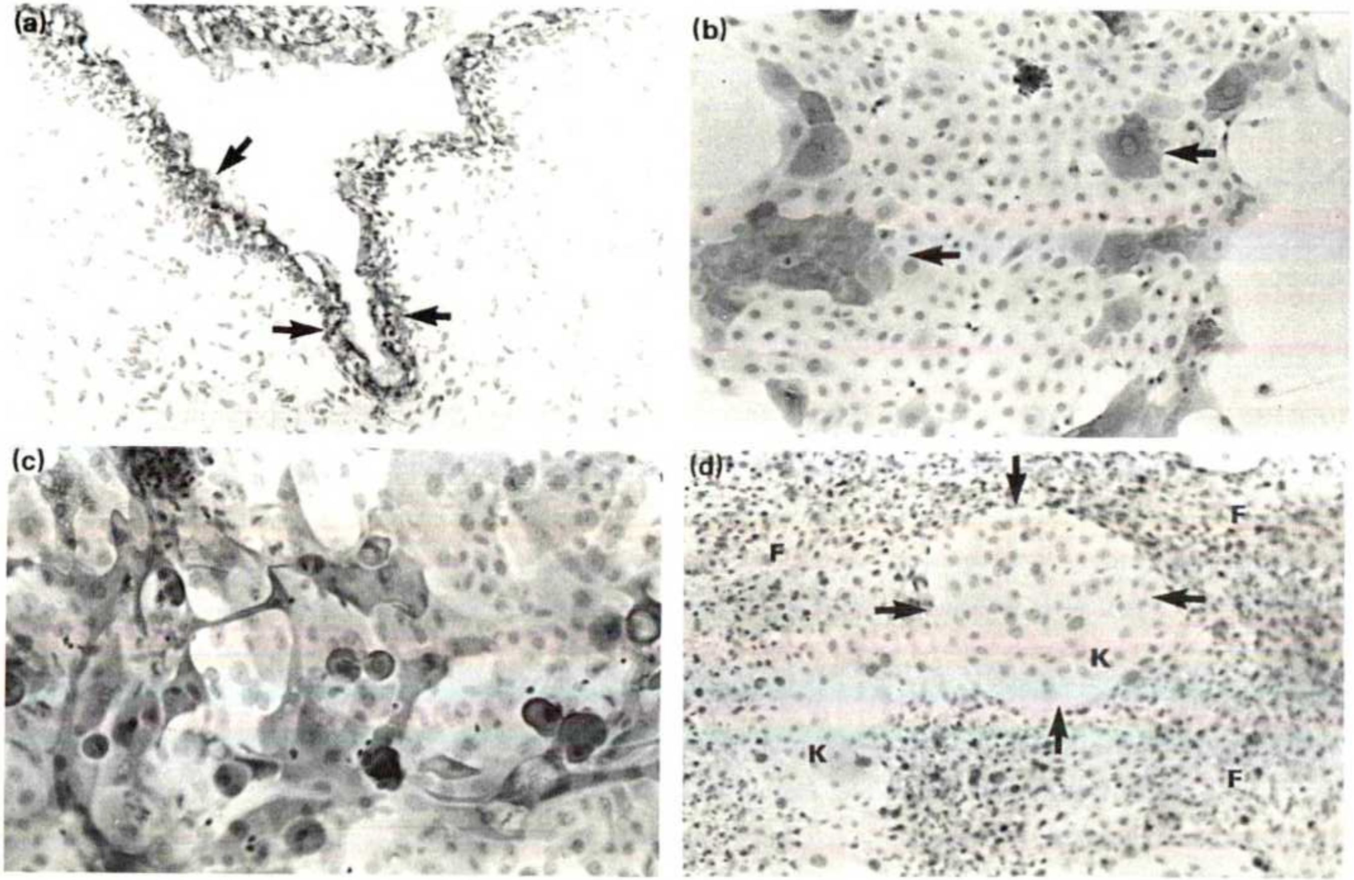

FIGURE 3. Staining pattern observed with the monoclonal antibody RPN1162 against cytokeratin 7. (a) Foetal skin at 14 weeks EGA, × 200; (b) foetal cells in 3T3 feeder after 4 weeks in culture, × 100; (c) adult cells after 3 weeks in culture, × 200; (d) negative staining of 3T3 cultured foetal keratinocytes for cytokeratin 8 after 2 weeks in culture, × 100. Note the small negative colony of keratinocytes (K) surrounded by 3T3 fibroblasts (F).

TABLE 3. The differentiation and proliferation markers of cultured keratinocytes

Antibody	Foetal skin	Culture	Adult skin	Culture
Vimentin	–	+ +	–	+ +
Transferrin receptor	–	+ + +	–	+ + +
Ki67	–	+ + +	+	+ + +
Involucrin	+ +	+ +	+ +	+ +
GB5 (suprabasal)	+ +	+ +	+	+
GB6 (basal)	+ +	+	+	+
EGF-r	+	+ +	+ +	+ +
Class I	–	+	+ +	+ +
Class II (Langerhans cells)	+	–	+ +	–

–, Negative staining.
+, Occasional positive cells present.
+ +, Positive cells consistently present.
+ + +, Abundant positive cells present.

observed *in vivo* and become indistinguishable from the adult cells *in vitro*. For example, the antibody RPN1164 reacts with the majority of basic type II cytokeratins including cytokeratin 8.[11] In foetal samples over 15 weeks EGA weak suprabasal staining is observed with this antibody, while adult skin is negative. Previous studies[12,13] have shown type II cytokeratin expression prior to 15 weeks EGA and this discrepancy may be due to the different antibodies these workers used. However, in culture the majority of keratinocytes stained strongly with this antibody. This may indicate the simple epithelial nature of the cultured keratinocyte and points to the modification of the cell by the tissue culture environment. Similarly cytokeratin 18, normally only found in simple epithelia and Merkel cells[13] is observed in culture. The fact that other workers[7] did not show cytokeratin 18 expression in culture could be due to the different antibody and techniques utilized. Cytokeratins 7 and 19, weakly expressed in foetal skin and absent in the adult, were also strongly expressed on a small percentage of cultured cells. This agrees with previous work on cytokeratin 19[14] although cytokeratin 7 has not been reported before. These altered cytokeratin patterns are probably a reflection of the changing nature of the keratinocyte, for example during differentiation basal cytokeratins disappear to be replaced by those of the suprabasal layers.[15]

Cultured cells were also shown to express vimentin (a mesenchymal marker) that is strongly expressed on fibroblasts. Co-expression of cytokeratin and vimentin has been previously observed in cultured adult cells[16,17] and may be related to the proliferative state of the cell, or to the adaptation of cells to the culture conditions. The proliferation markers, Ki67 and transferrin receptor, were also strongly expressed on cultured cells, as were cytokeratins 13 and 16. It is interesting to note that although cytokeratin 13 and 16 are expressed in foetal skin, Ki67 and transferrin receptor are not. The association of cytokeratin 13 with wet mucosal surfaces[18] and submerged keratinocyte cultures may partly account for the expression observed in foetal skin and cultured cells. Cytokeratin 13 and 16 are expressed in hyperproliferative states such as psoriasis[4,13] (personal observation), and squamous cell carcinomas[19] although not in basal cell epitheliomas,[18] indicating that different states of proliferation may exist.

Involucrin is the precursor of the cross-linked envelope that forms during terminal differentiation of the keratinocyte[20] and has a different pattern of distribution in the foetus and adult. However, in culture a small number of cells were positive on 3T3 confluent cell cultures, indicating that foetal cells eventually differentiate.

In summary, this report characterizes foetal keratinocytes in culture and indicates that the cytokeratin profile can be influenced by tissue culture conditions and is not merely a reversion of the adult to the foetal prototype.

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Expression of extracellular matrix in hair follicle mesenchyme in alopecia areata

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SUMMARY

Human hair follicle mesenchyme contains a distinctive extracellular matrix which varies in volume and composition in concert with the hair growth cycle. To investigate the possibility that mesenchymal function is disturbed in alopecia areata we have studied the expression of extracellular matrix constituents in scalp biopsies from 14 patients by immunohistochemical staining for basement membrane proteins, proteoglycans and interstitial collagens. The staining patterns in follicles from non-lesional scalp were normal. Miniature anagen follicles from bald patches also showed relatively normal expression of basement membrane proteins and proteoglycans. However, in some large anagen follicles from lesional sites, there was loss of the normal staining pattern for chondroitin-6-sulphate in the dermal papilla. In lesional catagen follicles, the glassy membranes showed marked convolution and thickening. These structures stained strongly for laminin and type IV collagen but only weakly for interstitial collagens.

Histological and ultrastructural studies in alopecia areata have demonstrated injury to cells in the precortical matrix region of lesional anagen follicles.[1,2] These cells also frequently display aberrant expression of class I and class II antigens of the major histocompatibility complex (MHC).[3,4] This appears to be a late event and may result from cytokine release by infiltrating immune cells.[5] These observations led us to suggest that cells undergoing early cortical differentiation form the primary disease target within the hair follicle.[2]

An alternative hypothesis is that epithelial changes in the hair follicle are a consequence of dysfunction of the dermal papilla. This is supported by the results of morphometric and autoradiographic studies.[6,7] The dynamics of the hair-growth cycle are disturbed in alopecia areata,[8] and it is known from experimental evidence in the rat that the dermal papilla is of crucial importance in controlling growth and differentiation of hair bulb epithelium.[9,10]

Relatively little is known of the molecular processes involved in signalling between the mesenchyme-derived dermal papilla and the epithelial matrix of the hair follicle. However, there is evidence to suggest that the extracellular matrix within the mesenchymal compartment

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plays a significant role in hair growth control. The extracellular matrix of the dermal papilla differs in content from that of the interfollicular dermis, being rich in basement membrane proteins and chondroitin proteoglycans. Moreover, in normal follicles, the volume and constitution of this extracellular matrix shows regular variation with the phase of the hair-growth cycle. During anagen, the dermal papilla cells are separated by an abundant extracellular matrix. The volume of the extracellular matrix diminishes during catagen and is minimal during telogen when the dermal papilla consists of a tightly packed ball of cells in close apposition with the secondary epithelial germ. Production of extracellular matrix resumes when the follicle re-enters anagen.

To investigate correlations of the pathophysiology of alopecia areata with extracellular matrix expression, we performed an immunohistochemical study using a panel of antibodies against basement membrane proteins, proteoglycans and interstitial collagens on biopsies from patients with various stages of the disease.

METHODS

Materials

Details of the antibodies used for immunohistochemical staining are summarized in Table 1. Biotinylated secondary antibodies and avidin–biotin–peroxidase complex were purchased from Vector Laboratories and chondroitin ABC lyase from ICN Biomedicals. The chondroitin and dermatan antibodies recognize epitopes revealed by prior digestion of glycosaminoglycans with chondroitin ABC lyase.[11] The antibody to dermatan sulphate also recognizes chondroitin-4-sulphate.

Tissue sections

Fourteen scalp biopsies were obtained with informed consent from patients with alopecia areata (Table 2). Normal scalp for control purposes was obtained from two patients during routine excision of benign tumours. Biopsy specimens were snap frozen, embedded in OCT medium and stored at −70°C until required. Sections 6 μm thick were cut on a cryostat, mounted on poly-l-lysine coated slides, air-dried and fixed in acetone.

Immunohistochemistry

Tissue sections were washed in phosphate-buffered saline (PBS) and those for proteoglycan staining were pre-incubated at 37°C with chondroitin ABC lyase (0·2 U/ml) for 30 min. Serial

TABLE 1. Antibodies used for immunohistochemical staining

Specificity	Species	Type	Dilution	Supplier
Type I collagen	Goat	Monoclonal	1:100	Sera-Lab Ltd
Type III collagen	Goat	Monoclonal	1:100	Sera-Lab Ltd
Type IV collagen	Mouse	Monoclonal	1:100	ICN Biomedicals
Laminin	Rabbit	Polyclonal	1:100	Unilever research
Unsulphated chondroitin (1B5)	Mouse	Monoclonal	1:100	ICN Biomedicals
Chondroitin-6-sulphate (3B3)	Mouse	Monoclonal	1:100	ICN Biomedicals
Dermatan sulphate (9A2)	Mouse	Monoclonal	1:100	ICN Biomedicals

TABLE 2. Clinical features of patients included in the study

Age	Sex	Duration	Biopsy Site	Type
26	F	4 years	Occipital	Non-lesional
32	F	12 years	Parietal	Non-lesional
22	M	10 months	Temporal	Pigmented regrowth
27	M	8 months	Occipital	Pigmented regrowth
26	F	4 years	Occipital	Edge of active patch
56	F	51 years	Occipital	Edge of active patch
57	M	9 months	Occipital	Edge of active patch
60	M	18 months	Occipital	Edge of active patch
19	F	13 years	Temporal	Bald area
39	M	3 years	Occipital	Bald area
21	M	20 years	Occipital	Non-pigmented regrowth
38	F	6 months	Occipital	Non-pigmented regrowth
39	F	9 months	Parietal	Non-pigmented regrowth
46	F	15 years	Temporal	Non-pigmented regrowth

incubation was then performed with blocking serum, primary antibody, biotinylated secondary antibody and avidin–biotin–peroxidase complex. All incubations were for 30 min with three 5 min washes in PBS containing 1% bovine serum albumin between each stage. The peroxidase reaction was developed with 0·05% diaminobenzidine and hydrogen peroxide 0·01% in 0·05 M Tris buffer at pH 7·6. The sections were lightly counterstained with haematoxylin, dehydrated and mounted. Controls included replacing the primary antibody with PBS or irrelevant antibodies and omission of chondroitin ABC lyase digestion where appropriate.

RESULTS

Scalp from normal subjects, non-lesional alopecia areata and pigmented hair regrowth

Staining patterns in these biopsies were identical to those previously noted in normal scalp.[12] In anagen follicles, there was staining throughout the dermal papilla with all the antibodies. The antibodies to collagen type I, collagen type III and dermatan sulphate also stained in the connective tissue sheath and throughout the interfollicular dermis. The antibodies to non-sulphated chondroitin and chondroitin-6-sulphate stained in the lower part of the connective tissue sheath, while those to type IV collagen and laminin stained the follicular basement membranes. During follicular regression in catagen, expression of proteoglycans declined with loss of non-sulphated chondroitin and chondroitin-6-sulphate preceding that of dermatan sulphate. By the telogen phase, the volume of matrix in the dermal papilla was greatly diminished. Expression of proteoglycan molecules was not apparent and that of basement membrane components was minimal at this stage. Typical patterns of staining for type I collagen, laminin and chondroitin-6-sulphate in anagen follicles are shown in Figure 1.

Margins of extending lesions

Biopsies from the margins of extending areas of alopecia areata contained a high proportion of catagen follicles. In one biopsy where surviving anagen follicles showed a heavy peribulbar

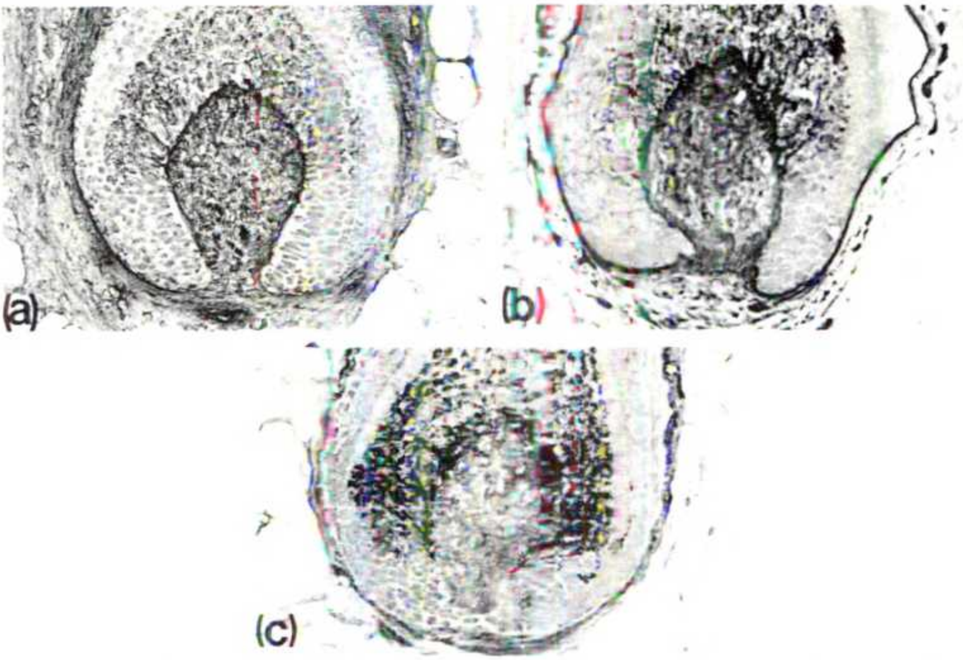

FIGURE 1. Staining of non-lesional anagen hair bulbs. (a) Type I collagen showing staining throughout the dermal papilla and connective tissue sheath. (b) Laminin showing staining in the basement membranes of the outer root sheath and blood vessels and throughout the dermal papilla. (c) Chondroitin-6-sulphate showing staining of the matrix of the dermal papilla and connective tissue sheath. Magnification × 142.

lymphoid infiltrate, there was loss of staining for chondroitin-6-sulphate in the upper half of the dermal papilla and the lower connective tissue sheath. In some catagen follicles, a greatly thickened and convoluted glassy membrane was noted. The glassy membrane showed intense staining for collagen type IV and laminin but stained only weakly with the antibodies to type I and type III collagen (Figs 2a and b). Staining for chondroitin-6-sulphate was restricted to a small rim at the outer margin of the glassy membrane (Fig. 2c). In some follicles, remnants of the glassy membrane were left beneath the hair bulb as the level of this structure rose in the dermis with the approach of telogen (Fig. 2d). Staining for collagen types I, III and IV and laminin was maintained in the dermal papilla during catagen but staining for non-sulphated chondroitin and chondroitin-6-sulphate was greatly diminished.

Bald areas and non-pigmented regrowth

In biopsies from established bald patches, numerous miniature anagen follicles were seen. The dermal papillae in these miniature follicles showed relatively normal patterns of staining with all the antibodies including chondroitin-6-sulphate (Fig. 3a).

Some anagen follicles in biopsies from areas of non-pigmented regrowth showed patchy staining or absence of staining from the upper pole of the dermal papilla for chondroitin-6-sulphate (Fig. 3b). Staining of dermal papillae in these follicles with other antibodies showed no gross abnormalities. However, some abnormalities of follicular anatomy were clearly demon-

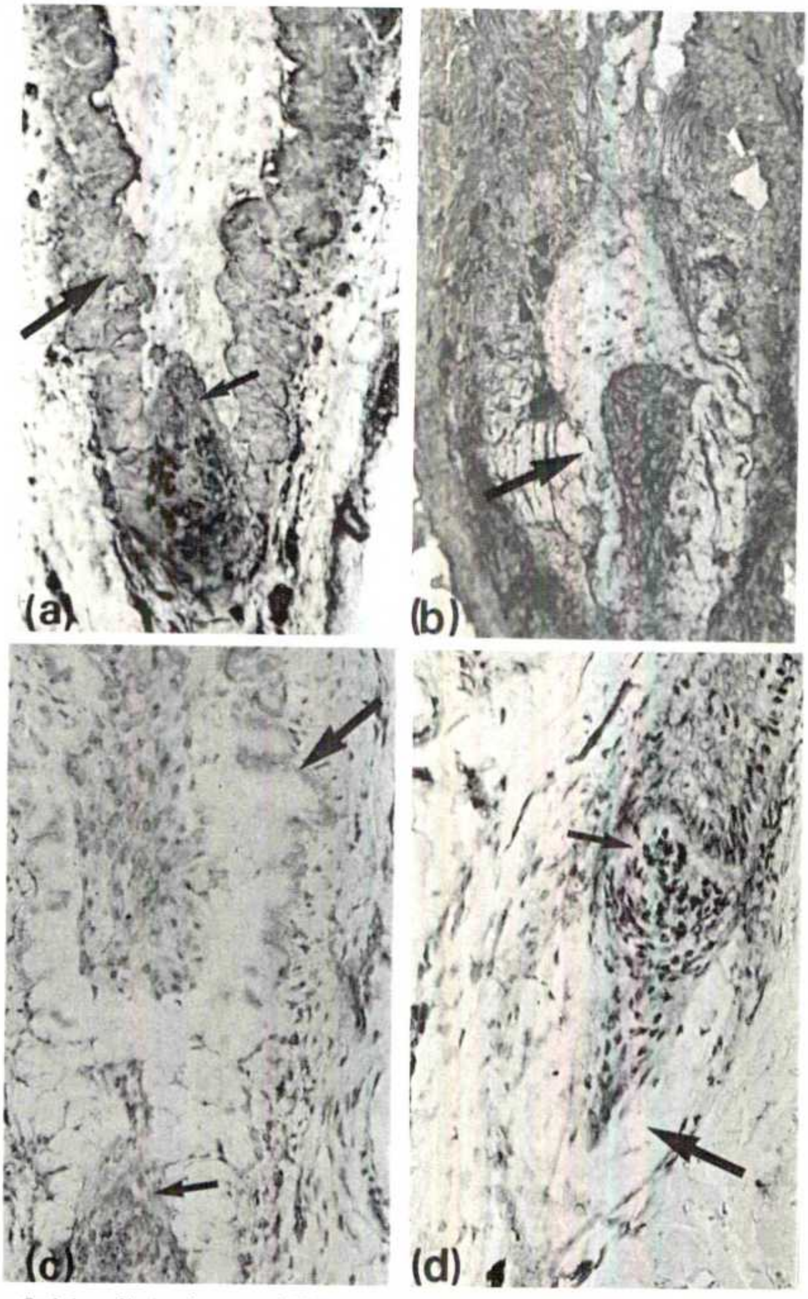

FIGURE 2. Staining of lesional catagen follicle. (a) Type IV collagen showing intense staining of the glassy membrane (thick arrow) and dermal papilla (thin arrow). (b) Type III collagen showing dense staining of the dermal connective tissues and dermal papilla while the glassy membrane (arrow) is virtually unstained. (c) Chondroitin-6-sulphate showing staining confined to a narrow rim (thick arrow) at the periphery of the glassy membrane and very weak in the dermal papilla (thin arrow). (d) Chondroitin-6-sulphate staining of lesional late catagen/telogen follicle showing unstained remnants of the glassy membrane (thick arrow) beneath the dermal papilla (thin arrow). The papilla is only weakly positive. Magnification: × 198.

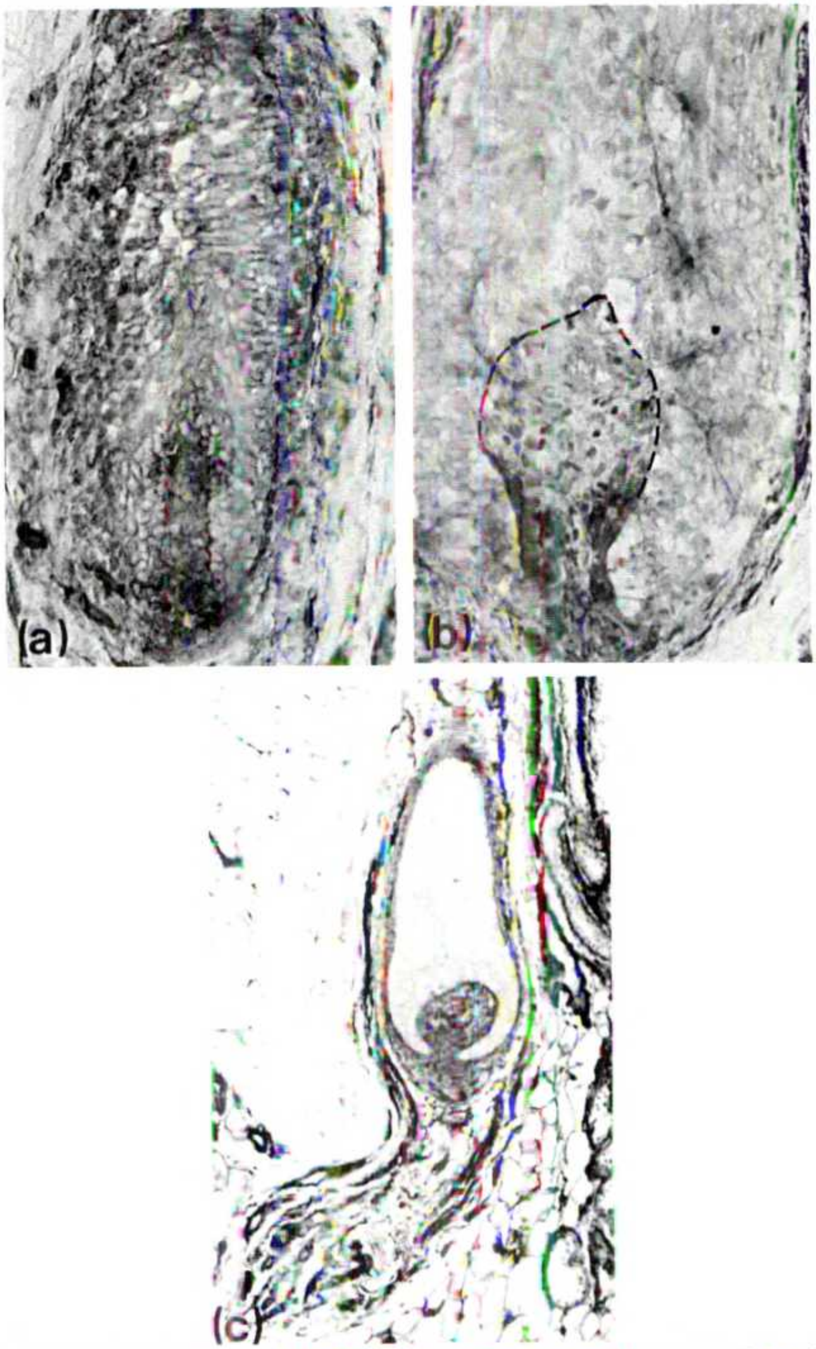

FIGURE 3. (a) Miniature anagen follicle from bald scalp showing normal expression of chondroitin-6-sulphate in the dermal papilla. Magnification: × 198. (b) Lesional anagen follicle from area of non-pigmented regrowth showing absence from the upper pole of the dermal papilla of chondroitin-6-sulphate. The papilla is delineated by the dotted line. Magnification: × 320. (c) Lesional follicle from area of non-pigmented regrowth showing relatively large dermal papilla with fibrous tracts extending into the dermis beneath (type IV collagen stain). Magnification: × 80.

strated, including fibrous tracts streaming beneath follicles situated unusually high in the dermis (Fig. 3c).

All the controls were negative.

DISCUSSION

The expression of basement membrane proteins and proteoglycans during hair follicle development and in the hair-growth cycle has been studied in the rat. Laminin and type IV collagen are present throughout the hair cycle[13] though they are most abundant in association with anagen follicles. Expression of fibronectin, heparan-sulphate proteoglycan and chondroitin-sulphate proteoglycan all show correlation with the phase of the hair growth cycle.[13-15] This is most marked for chondroitin-sulphate proteoglycan and it has been suggested that this basement membrane component may be involved in the dermo-epidermal interactions leading to formation and growth of skin appendages.[15] Moreover, in chick embryo tissue culture experiments, disturbed proteoglycan metabolism has been found to result in morphogenetic abnormalities of feather follicles.[16]

Expression of proteoglycans during the human hair cycle has recently been studied in normal scalp with antibody techniques similar to those we have used.[12] Similar findings of a cyclical pattern of expression of chondroitin proteoglycans have emerged; staining for non-sulphated chondroitin and chondroitin-6-sulphate declines in the dermal papilla and connective tissue sheath early in catagen and becomes undetectable by telogen. These changes appear to occur earlier than the decline in expression of other proteoglycans or basement membrane proteins. Involvement of proteoglycans in human hair-growth regulation had previously been inferred from observations in diseases such as pretibial myxoedema and the Hunter/Hurler syndrome in which disturbed proteoglycan metabolism is associated with hypertrichosis.[17,18]

In newly involved areas of alopecia areata, anagen follicles are precipitated abruptly and prematurely into catagen. The unusual patterns of staining for chondroitin-6-sulphate in some anagen follicles from lesional sites may indicate impending catagen conversion. It was apparent that these changes were only in follicles where entry into catagen was likely to be imminent. However, loss of chondroitin-6-sulphate staining is not usually observed before the occurrence of early morphological changes of catagen. It is possible that the abnormal appearances in these lesional anagen follicles was due to degradation of extracellular matrix by infiltrating inflammatory cells. Nevertheless, we cannot exclude the possibility that these changes indicate primary dysfunction of the hair follicle mesenchyme with resulting premature entry of follicles into catagen.

The grossly thickened and convoluted appearance of the glassy membrane in lesional catagen follicles has, to our knowledge, not been previously reported. We have not encountered similar appearances in catagen follicles from normal subjects. The role of the glassy membrane in the evolution of catagen is unknown and the significance of this observation is not clear. Nevertheless, these findings provide confirmation that the glassy membrane is composed of basement membrane proteins. Chondroitin-6-sulphate has been regarded as a basement membrane component[19] and has recently been suggested to be important in the pathogenesis of dystrophic epidermolysis bullosa.[20] It is interesting, therefore, that staining for this proteoglycan did not co-distribute with laminin and type IV collagen in catagen follicles.

In conclusion, the changes we have observed in alopecia areata may result from dynamic disturbances of the hair growth cycle. However, the loss of the normal staining pattern for chondroitin-6-sulphate in some lesional anagen follicles suggests that the changes which occur in the dermal papilla during normal catagen are occurring prematurely. This observation warrants further study and should be amenable to investigation by *in vitro* techniques.

ACKNOWLEDGMENT

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Silica-associated systemic sclerosis is clinically, serologically and immunologically indistinguishable from idiopathic systemic sclerosis

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SUMMARY

To determine whether the clinical, immunological and serological features of patients with silica-associated systemic sclerosis are different from patients with the 'idiopathic' form of systemic sclerosis (SS) we studied 22 underground coal miners who were exposed to silica dust (SD), 30 mine workers who later developed silicosis (S) and 17 mine workers exposed to silica dust who subsequently developed a systemic sclerosis-like disease (SA-SS).

The patients with SA-SS had features clinically indistinguishable from individual patients with SS. They all had Raynaud's phenomenon, 14 had cutaneous sclerosis identical to that seen in acrosclerosis and three had a generalized cutaneous sclerosis. Sixteen patients had bibasilar pulmonary fibrosis, 10 had necrosis of the fingertip pulps, nine had oesophageal involvement and only one patient had renal involvement. Antinuclear antibodies and circulating immune complexes were detected in three and eight patients with SD, 14 and five patients with S and in 16 and nine patients with SA-SS, respectively. Anti-Scl-70 antibody was detected in eight of the 17 patients with SA-SS. Evidence for *in vivo* endothelial cell damage, as determined by elevated levels of von Willebrand factor, was found in nine patients with SD, 14 patients with S and in 10 patients with SA-SS. Following incubation of the patient's serum with confluent cultures of human umbilical vein endothelial cells there was only a significant reduction in calcium ionophore-induced release of prostacyclin with the serum from SA-SS patients compared to that with control serum (NC). The mean ± SEM release of 6-keto-PGF$_{1\alpha}$ (the stable metabolite of prostacyclin expressed as ng/10^4 cells) decreased from $2{\cdot}90 \pm 0{\cdot}27$ to $2{\cdot}01 \pm 0{\cdot}33$ (SD),

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3·34 ± 0·42 to 1·76 ± 0·31 (S), 1·98 ± 0·12 to 0·64 ± 0·07 (SA-SS) and 2·28 ± 0·33 to 1·36 ± 0·21 (NC) with 1 and 20% serum, respectively.

This study demonstrates that immune complex and antinuclear antibody formation and *in vivo* endothelial cell damage occurs following occupational exposure to silica. The patients who subsequently develop a systemic sclerosis-like disease have clinical, immunological and serological features which are indistinguishable from the idiopathic form of the disease although as a group the SA-SS patients have a higher prevalence of pulmonary involvement and the anti-Scl-70 antibody.

Systemic sclerosis-like disorders have been reported to develop following exposure to vinyl chloride,[1] aliphatic[2] and aromatic[3] hydrocarbons, epoxy resins,[4] silicon,[5] bleomycin[6] and toxic oil.[7] Bramwell first drew attention to the possible link between occupational silica-dust exposure and systemic sclerosis when he noticed a much higher incidence of this disease in his practice in Edinburgh compared to the incidence reported in the literature.[8] Surprisingly, there was only one female in his nine patients with systemic sclerosis and the males had been occupationally exposed to silica either as stonemasons, as a miner or as a copper smith. Forty years later Erasmus expressed concern about the high prevalence of systemic sclerosis in gold miners examined at The Silicosis Medical Bureau in Johannesburg.[9] Subsequently, there have been many reports describing the association of systemic sclerosis with either silica exposure or silicosis in Italy, France, Germany, South Africa and Japan.[10–15] Epidemiological studies have shown an almost threefold increase in incidence of systemic sclerosis among coal miners compared to a large control group, a 25-fold higher risk of developing a systemic sclerosis-like disorder in work places where there is exposure to silica and the probability of developing this disease is 110 times higher for patients with silicosis than for the normal male population.[16,17]

There is evidence that in some of the exogenously-induced diseases, particularly that caused by vinyl chloride, the systemic sclerosis-like features improve or resolve when the patient is removed from the causative agent.[18] Although there have been occasional reports of spontaneous remission in idiopathic systemic sclerosis[19] no such event has been documented in silica-induced systemic sclerosis. This raises the question as to whether the two diseases are clinically indistinguishable and represent a similar host response to a variety of agents. We therefore studied the clinical, immunological and serological features of miners exposed to silica dust who developed either silicosis, a systemic sclerosis-like disorder or no associated disease to determine whether there were any characteristic differences.

METHODS

Subjects

Twenty-two male underground coal or uranium workers (mean age 47 years, range 27–68) with a minimum of 5 years' exposure to silica dust (range 5–15) who were regularly followed up in an occupational health clinic and were otherwise healthy and had normal annual chest radiographs were selected. Thirty male miners (mean age 58 years, range 47–68) who were diagnosed as having silicosis on radiographic criteria or in problematical cases by examination of scalene lymph nodes for silicotic nodules, and 17 male workers (mean age 58 years, range 47–50) with silica-associated systemic sclerosis were recruited. Nine healthy controls (mean age 44 years, range 23–65) who had no past history of silica exposure and who had no evidence of a connective tissue disease were also selected. Serum was collected from all the patients and controls and was stored at -20°C until later used. Clinical details were collected by three of the authors (V.Z., U-F. H and P.M.D).

Measurement of von Willebrand factor

Ninety-six-well microtitre plates (Nunc, U.K.) were coated with 1/1000 anti-human von Willebrand factor antibody (vWF) (Dako, U.K.) and test serum, diluted 1/40 with phosphate-buffered saline (PBS, pH 7·4), was added and incubated for 3 h. The wells were washed with PBS and then anti-human vWF peroxidase conjugate diluted 1/1000 with PBS was added. After further washing with PBS the substrate 1,2 *o*-phenylenediamine dihydrochloride (Sigma, U.K.) was added and the reaction was read at a wavelength of 492 nm. Units of vWF were expressed as a percentage of serum from healthy volunteers and those results greater than 2 SD from a large control sample (i.e. $> 120\%$) were considered raised.

Endothelial cell cultures

Human umbilical vein endothelial cells were grown in culture using the method described by Jaffe *et al.*[20] Briefly, umbilical veins from individual cords were cannulated, washed with balanced-salt solution (BSS, pH 7·4) and filled with 0·1% collagenase (Clostridium Histolyticum, Boehringer Mannheim GmbH, F.R.G.). After a 12-min incubation at 37°C, detached cells were obtained by flushing with Medium 199 and were centrifuged at 250 ***g*** for 5 min. The cell pellet was resuspended in M199 containing 100 U/ml penicillin, 50 μg/ml streptomycin, amphotericin B, glutamine (Gibco) and 20% pooled human serum and were grown in 24-well plastic cluster plates (Sterilin Ltd, Feltham, England) in humidified 5% CO_2/95% air at 37°C. In all experiments the cells used were primary cultures at confluence and they were identified as endothelial cells by their 'cobblestone' appearance and by positive uniform staining for von Willebrand factor.

Incubations of cultured endothelial cells with serum

Confluent primary cultures were washed once with M199 and then were incubated with either 1 or 20% serum from each patient for 24 h in humidified 5% CO_2/95% air at 37°C. At the end of each incubation the supernatant was removed, the cells were washed with M199 and stimulated with the calcium ionophore A23187 (10 μM) (Sigma, Poole, Dorset, U.K.) for 2 h. The supernatants were collected and frozen at -20°C for subsequent radioimmunoassay of 6-keto-$PGF_{1\alpha}$. The endothelial cells were trypsinized (0·25% trypsin), centrifuged at 500 ***g*** for 1 min, resuspended in 200 μl of BSS and after addition of 10 μl of methylene blue the cells were counted in an improved Neubauer Chamber (Weber, England). The mean number of endothelial cells was 1×10^5 per well.

Radioimmunoassay of 6-keto-$PGF_{1\alpha}$

The antibody to 6-keto-$PGF_{1\alpha}$ (donated by Dr M. Greaves, Department of Haematology, Royal Hallamshire Hospital, Sheffield, U.K.) was incubated with the samples overnight at 4°C. Cross reactivities of the antiserum with other major eicosanoids (PGE_2, $PGF_{2\alpha}$, TXB_2) were less than 1%. The tracer was 6-keto-[^{3}H]$PGF_{1\alpha}$ (Amersham International PLC, Amersham, U.K.) and the standard was 6-keto-$PGF_{1\alpha}$ (Wellcome Laboratories, Beckenham, U.K.). Unbound tracer was separated from bound by the addition of dextran/charcoal. The assay was linear over the concentration range 0·1–2·5 ng/ml and the limit of sensitivity was 39 pg/ml.

Measurement of circulating immune complexes

Circulating IgG immune complexes were measured using the polyethylene glycol (PEG)-precipitation method.[21] Briefly, samples of serum were incubated with 2% PEG (Sigma, Dorset, U.K.) for 18 h at 4°C and after centrifugation the precipitate was washed with 2% PEG and then with PBS. Samples of dissolved precipitate were run on single-layer immunodiffusion agar plates against anti-IgG and the diameters were measured after 48 h. Results were compared with standards containing known quantities of immune complexes.

Statistical analysis

Differences in prostaglandin release from cells pre-exposed to the different sera were analysed by comparing the effect of one serum concentration from patients with SA-SS, SD or S against the same concentration from normal individuals using the Mann–Whitney *U*-test. $P < 0{\cdot}05$ was considered to be significant.

RESULTS

The 17 patients with silica-associated systemic sclerosis had been exposed to silica while working either as a miner ($n = 15$), as a stove-fitter ($n = 1$) or as a sandblaster ($n = 1$) for a mean length of 16·2 years (range 4–36). The interval between the end of exposure and the onset of the features of systemic sclerosis ranged from 0 to 28 years with a mean of 11·2 years. The patients had features of systemic sclerosis for a mean of 6 years (range 1–15) and all had Raynaud's phenomenon, 16 had bibasilar pulmonary fibrosis, 14 had a limited acral dermal sclerosis, three had generalized or diffuse dermal sclerosis, 10 had necrosis of the fingertip pulps, nine had oesophageal involvement and only one had renal involvement (Table 1). The interval between the onset of their Raynaud's phenomenon and the cutaneous sclerosis was a mean of 3 years (range 0–10). Because of a change in mining conditions during 1960–61 when dust control standards were improved and levels of silica dust in the air were dramatically reduced, patients exposed to silica dust were subdivided into two groups, those exposed between 1945 and 1960 and those exposed after 1961.

Detectable concentrations of circulating immune complexes (CIC) measured by the polyethylene glycol precipitation method were found in all of the pre-1961 SD miners and in over 50% of the SA-SS patients (Table 2). Raised levels of immunoglobulins were mainly detected in the SA-SS patients and in no person were reduced levels of C3 or C4 found. Using HEp-2 cells as substrate, antinuclear antibodies were detected in three patients with exposure to silica dust between 1945 and 1960 (two grainy and one fine speckling pattern), in none of the

TABLE 1. Clinical features of the 17 patients with silica-associated systemic sclerosis

Raynaud's phenomenon	$n = 17$
Limited dermal sclerosis	$n = 14$
Generalized dermal sclerosis	$n = 3$
Necrosis of finger tip pulp	$n = 10$
Bibasilar pulmonary fibrosis	$n = 16$
Oesophageal involvement	$n = 9$
Renal involvement	$n = 1$

TABLE 2. Circulating immune complexes, immunoglobulin and complement levels in the patient groups

	SD < 1961	SD > 1961	S	SA-SS
CIC	8/8	3/12	5/30	9/17
↑ IgG	1/8	2/12	3/30	9/17
↑ IgM	0/8	1/12	2/30	3/17
↑ IgA	1/8	2/12	5/30	7/17
↓ C4	0/8	0/12	0/30	0/17
↓ C3	0/8	0/12	0/30	0/17

patients exposed to silica dust after 1961, in 14 patients with silicosis (eight nucleolar, two homogeneous and four fine speckling pattern) and in 16 patients with silica-associated systemic sclerosis (eight anti-Scl-70, two anticentromere (ACA), two anti-RNP and four nucleolar) (Table 3). Elevated levels of von Willebrand factor were found in nine of 22 SD patients (41%), 12 of 30 S patients (40%) and in 10 of 17 patients with SA-SS (59%) (Table 4).

There was a concentration-dependent inhibition of the release of 6-keto-PGF$_{1\alpha}$ by serum from all the patients following a 24-h incubation with the cultured endothelial cells (Fig. 1). The

TABLE 3. Antinuclear antibodies in different patient groups using immunofluorescence of HEp-2 cells and immunodiffusion techniques

	SD < 1961	SD > 1961	S	SA-SS
Speckled	1	—	4	—
Nucleolar	—	—	8	4
Grainy	2	—	—	—
Homogeneous	—	—	—	—
Anti-RNP	—	—	—	2
Anti-Scl-70	—	—	—	8
ACA	—	—	—	2

TABLE 4. Levels of serum von Willebrand factor in the patient groups

		vWF expressed as % of normal serum				
		< 120	120–140	140–180	180–400	> 400
SD 1945–60	$n=8$	4	0	1	1	2
SD 1961–83	$n=14$	9	0	2	1	2
S	$n=30$	18	0	1	1	10
SA-SS	$n=17$	7	3	4	3	0

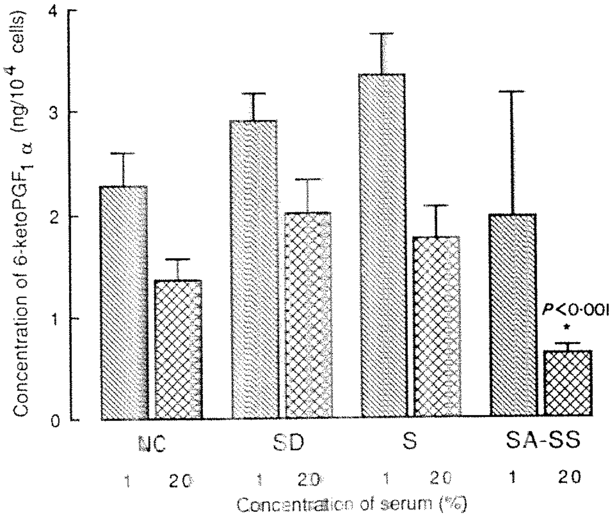

FIGURE 1. Effect of different concentrations of serum from normal controls (NC), miners exposed to silica dust (SD), miners who developed silicosis (S) and miners who developed silica-associated systemic sclerosis (SA-SS) on the release of 6-keto-PGF$_{1\alpha}$ by cultured human umbilical vein endothelial cells.

mean ± SEM release of 6-keto-PGF$_{1\alpha}$ (ng/10^4 cells) decreased from 2·90 ± 0·27 to 2·01 ± 0·33 (SD), 3·34 ± 0·42 to 1·76 ± 0·31 (S), 1·98 ± 0·12 to 0·64 ± 0·07 (SA-SS) and 2·28 ± 0·33 to 1·36 ± 0·21 (NC) with 1 and 20% serum, respectively. Compared to the control serum, only with the 20% SA-SS serum was there a significant inhibition ($P < 0·001$). There was no significant difference between the values obtained with serum from either the SD or S patients compared to the control serum.

DISCUSSION

This study has shown that patients with silica-associated systemic sclerosis had clinical features indistinguishable from those of idiopathic systemic sclerosis and that they satisfy the standard criteria for the diagnosis of systemic sclerosis.[22] This is in contrast to a previous suggestion that such patients should be regarded as having pseudoscleroderma because Raynaud's phenomenon, microstomia and systemic involvement had been absent and the skin disease was often localized.[23] As a group, the SA-SS patients had a higher prevalence of bibasilar pulmonary fibrosis and the presence of the anti-Scl-70 antibody, being found in 94 and 47% of patients, respectively. The mean prevalence of radiological lung involvement in 30 series totalling 1620 patients with systemic sclerosis was 36% and in 78 patients with the CREST variant the prevalence was only 19%.[24] The anti-Scl-70 antibody occurs in 34–77% of patients with diffuse systemic sclerosis and is usually associated with severe interstitial lung disease.[25,26] Perhaps therefore in genetically susceptible people who are exposed to silica there is increased production of the anti-Scl-70 antibody. Instead of the double association with diffuse cutaneous involvement and severe pulmonary involvement only the latter occurs, because only three of the 17 patients had diffuse dermal sclerosis. Clearly a larger number of patients with SA-SS need to be studied to see whether this is true.

In support of a genetic susceptibility, a survey of a large number of fluospar miners and their families in Sardinia suggested that some persons were predisposed to develop silicosis and others were not.[27] Furthermore, Bernstein and colleagues showed a highly significant increase in unstable C″ cells and aneuploid cells as well as sister chromatid exchanges in male goldminers who had systemic sclerosis.[28] Similar findings have been detected in patients with systemic sclerosis who had not been occupationally exposed.[29] It would be interesting to repeat these investigations in the SD and S mining groups to see whether the association only occurs in the patients who develop SA-SS. So far no HLA studies have been performed in patients with SA-SS but one study detected a decreased frequency of B7 in patients with silicosis and the authors suggested that this might be a predisposing factor.[30] Again further HLA studies are required in patients with SA-SS.

It is clear from the literature that there are several countries, such as South Africa, G.D.R. and Italy, from which many reports of SA-SS arise. There are also other countries such as the U.K. from which there have been no further reports of SA-SS since Bramwell's original description. For example, Haustein and colleagues assessed all the 237 patients living in the G.D.R. diagnosed as having systemic sclerosis and found that 66 of the 86 male patients had been exposed to silica dust and that 50% of these patients also suffered from silicosis.[17] In contrast Ruettner only found four cases of systemic sclerosis in 2000 patients with silicosis and not a single case of systemic sclerosis in 650 patients with silicosis who underwent autopsy.[31] It appears therefore that a variable proportion of workers occupationally exposed to silica do develop silica-associated systemic sclerosis and although in one report there was a suggestion that intensity of exposure was more important than duration of exposure,[32] there may be a complex interplay between environmental conditions and host factors that are as yet not understood.

Vascular damage is a recognized complication of systemic sclerosis and this is characterized by proliferative intimal arterial lesions and an obliterative microvascular process.[33,34] There was macroscopic evidence of these changes in the SA-SS patients with 59% having necrosis of the fingertip pulps. Unfortunately there is no totally satisfactory method for quantitating endothelial cell damage *in vivo* but it is known that disturbances of endothelial cell integrity *in vitro* can stimulate the release of von Willebrand factor protein.[35] It has been on the basis of this fact that circulating vWF has been measured in several diseases in which microvascular injury occurs. These include respiratory failure,[36] vasculitis,[37] diabetes mellitus[38] and gram negative septicaemia.[37] In this study raised levels of vWF were found in otherwise healthy miners exposed to silica dust, as well as in the patients with silicosis and SA-SS. Kahaleh *et al.* found elevated levels of vWF in 15 out of 17 patients with systemic sclerosis and in seven of nine patients with Raynaud's syndrome.[35] None of the healthy miners nor any of the miners with silicosis suffered from Raynaud's phenomenon and so it would be reasonable to suggest that exposure to silica and/or the development of the systemic sclerosis was the cause of the raised vWF levels in this study.

The cause of the microvascular damage in systemic sclerosis is not known but the presence of circulating immune complexes may be pathogenetic, and there was some correlation between presence of the immune complexes and increased levels of vWF in the patient groups in this study. However, there may be other factors responsible for such phenomena including anti-endothelial cell antibodies.[39] In order to investigate whether disturbed endothelial cell function occurred in the patients with SA-SS we used cultured human endothelial cells as a model. Serum from patients with SA-SS inhibited the release of prostacyclin from these cells and the effect was significantly greater than with control serum. This finding may be relevant to the pathogenesis of Raynaud's phenomenon in the patients with SA-SS since there may be a circulating factor(s) which impairs the release of the vasodilator and anti-aggregatory prostanoid PGI_2 from vascular endothelium following the vasoconstriction induced by cold. Similar findings were previously reported in patients with the idiopathic form of systemic sclerosis,[40] although other investigators found that incubation of serum from patients with systemic sclerosis did not cause any alteration in prostacyclin release from unstimulated microvascular endothelial cells.[41]

There is also a possibility that endothelial cells may be involved in stimulating the increased collagen synthesis that occurs in silicosis and SA-SS. It is known that silica particles are ingested by macrophages in the lungs and that silica exposure enhances release of interleukin 1 from alveolar macrophages.[42] Silica crystals also may be taken up by the lymphatics and following moderate but prolonged exposure silicotic islets continue to proliferate in both the lung parenchyma and the lymph glands. The involvement of the lymph glands can extend beyond the hilum of the lungs and silicotic nodules may be found in the cervical glands and along the aortic chain down to the inguinal glands.[43] It would be reasonable to hypothesize that with such widespread dissemination of silica crystals that some enter the general circulation and that these are then phagocytosed by vascular endothelium. This is supported by the finding of silicotic nodules in the liver, spleen and bone marrow.[44–46] Endothelial cells that have phagocytosed silica could then be stimulated to release interleukin 1 as previously demonstrated by Miossec and colleagues[47] or platelet-derived growth factor or other factors which would stimulate fibroblast proliferation and collagen deposition. This theory would support the observation of Sluis-Cremer *et al.* that it is the intensity of exposure rather than the duration of working exposure that predisposes to SA-SS.[32] It would be interesting to see therefore whether cultured endothelial cells can phagocytose silica crystals and whether such a phenomenon can be demonstrated in pathological specimens. Furthermore it would be of interest to see whether unstimulated peripheral blood monocytes have an increased spontaneous release of interleukin 1, a finding previously reported in patients with systemic sclerosis having a disease history of less than 5 years.[48]

In conclusion, this study has shown that patients with silica-associated systemic sclerosis have clinical, immunological and serological features indistinguishable from those patients with idiopathic systemic sclerosis. More research is required however to understand the basis of an individual's genetic susceptibility to the environmental agent silica and the sequence of events that leads to the characteristic systemic and cutaneous lesions of systemic sclerosis.

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Production of antikeratin autoantibodies by hybrid spleen cells of naive mice

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SUMMARY

The mechanism of the occurrence of natural antikeratin antibodies in human sera was studied using hybrid spleen cells obtained from experimentally naive or from immunized mice. Antikeratin antibodies were detected by enzyme-linked immunosorbent assay (ELISA) in 5·9–9·5% of the culture supernatants of fused spleen cells taken from naive mice. When mice were immunized with keratins, the number of supernatants containing antikeratin antibodies was increased to eight out of 51 (15·7%). When immunized with non-keratin materials such as activated human T cells, adult T-cell leukaemia cell lysates, and human T-cell lymphotropic virus type-1 (HTLV-1), 16·7–20·8% of the supernatants were found to contain antikeratin antibodies by ELISA.

The antikeratin antibodies in the supernatants showed cytoplasmic staining of keratinocytes in human as well as mouse skin by indirect immunofluorescence. The antibodies reacted with extracted human epidermal keratins by dot-blot and Western blot analysis. Most antikeratin antibodies in the supernatants did not show cross-reactivity with exogenous antigens used for immunization and vimentin-type intermediate-sized filaments.

These findings demonstrate that B cells producing antikeratin antibodies are common in naive mice, and produce various types of antikeratin antibodies following specific activation with epidermal keratins and non-specific immunological stimuli.

Circulating antibodies against epithelial cytoplasmic antigens are known to occur in various disorders and in normal subjects.[1-5] It has been demonstrated[2-6] that most epidermal cytoplasmic antibodies are directed against keratins and that antikeratin antibodies are more commonly detected in human sera than previously appreciated. These antikeratin antibodies show various immunofluorescence staining patterns on keratinized epithelia, and recognize different keratin subunits by Western blot analysis. The occurrence of such antibodies is

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increased in patients with various dermatoses, in those with myeloma, following bone marrow transplantation and in those with neoplasms, rheumatoid arthritis, and collagen diseases.[1,6,7]

It has also been reported that various kinds of autoantibodies to other cytoskeletons and cellular components are detected in human sera.[7-8] Previous *in vitro* studies have shown that virus-transformed B cells or human hybridoma cells secrete autoantibodies against various cellular components including cytokeratins.[9-12] Antibodies to intermediate-sized filaments were found in sera taken from patients with infectious mononucleosis.[13] These findings suggest that antikeratin antibodies, like antibodies to other cytoskeletons, are autoantibodies and that the production of such antibodies is increased by immunological stimulation or B-cell transformation.

We investigated the production of antikeratin antibodies by fused spleen cells obtained from experimentally naive mice. We found an increased production rate of such antibodies in mice immunized with not only keratins but also non-keratin immunogens.

METHODS

Extraction of epidermal keratins

Normal human epidermis was separated from dermis by incubation with 0·5 M EDTA, pH 7·5, at 60°C for 1 min, and keratin proteins of whole epidermis were purified and extracted as previously described.[14] Keratins of trypsinized keratinocytes, the remnant stratum corneum, and callosities were extracted separately in a similar fashion. All the materials were analysed by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE).

Preparation of hybridomas

Spleen cells taken from 10-week-old, non-immunized BALB/c mice were fused with a mouse myeloma cell line, NS-1, in 50% polyethylene glycol. Alternatively, spleen cells from 10-week-old mice immunized intraperitoneally with keratins or non-keratin immunogens such as phytohaemagglutinin (PHA)-activated human T lymphocytes, cell lysates of an adult T-cell leukaemia (ATL) cell line, and human T-cell lymphotropic virus type-1 (HTLV-1) were fused in the same manner. The ATL cell lysates and HTLV-1 were originally prepared for the production of specific antibodies against ATL cells and HTLV-1 virus antigens. The fused cells obtained from one spleen were seeded in two microplates with 96 flat-bottomed wells (Nunc, Netherlands) and selected in HAT medium containing 1×10^{-4} M hypoxanthine, $1{\cdot}6 \times 10^{-5}$ M thymidine, and 4×10^{-7} M aminopterin. After 2 weeks of culture, the presence of antikeratin antibodies in the supernatant of each well was determined by enzyme-linked immunosorbent assay. The positive wells were then studied by immunofluorescence and Western blot analysis.

Enzyme-linked immunosorbent assay (ELISA)

Each well of the 96-well microplates was coated with 50 μl of keratin extract (10 μg/ml in bicarbonate buffer, pH 9·6), at 4°C overnight. The plates were washed with 0·05% Tween-20, phosphate-buffered saline (PBS), pH 7·4, air-dried at room temperature, and stocked at −20°C until use. The keratin-coated wells were incubated with the culture supernatants at room temperature for 2 h, washed four times with 0·05% Tween-20 in PBS, followed by incubation with peroxidase-labelled F(ab) fraction of goat anti-mouse immunoglobulins (Medical Biological Laboratories, Nagoya, Japan) at a dilution of 1:800. Control-culture media were obtained from the wells without growth of hybridomas. After washing, the peroxidase activity was determined by colorimetric reaction with 2,2′-azino-di-3-ethyl-benzthiazoline-sulphonate (ABTS). The optical density of each well was read at 405 nm.

Indirect immunofluorescence

The culture media containing antikeratin antibodies were studied by indirect immunofluorescence using cryostat sections of normal human skin and BALB/c mouse skin. In order to reduce the background reaction, fluorescein isothiocyanate-labelled goat anti-mouse immunoglobulin was used following the absorption of the conjugate with epidermal insoluble fractions as previously described.[2]

Dot-blot analysis

Purified keratins, a Nonidet P-40 (NP40) extract of whole epidermis, and a cell lysate of PHA-activated T cells were diluted with PBS at concentrations of 40–100 μg/ml. Three microlitres of each sample was spotted on nitrocellulose strips, and air-dried at room temperature for 30 min. The strips were saturated with 5% bovine serum albumin (BSA)/Tris-HCl, pH 7·4, and reacted with each of the following first antibodies: supernatants of hybridomas, a monoclonal antikeratin antibody (KL-1, Immunotech, Marseille), and control mouse IgG1 (Becton Dickinson, Mountain View, CA, U.S.A.). After washing, the nitrocellulose strips were reacted with the peroxidase-labelled anti-mouse immunoglobulins at a dilution of 1:800. The peroxidase activity was visualized by incubation of the strips with 0·05% diaminobenzidine tetrahydrochloride in the presence of 0·05% hydrogen peroxide.

Western blot analysis

The extracted epidermal keratins were separated by SDS–PAGE, and transferred to nitrocellulose paper at 4°C for 3 h.[15] The strips taken from the nitrocellulose paper were incubated with 5% BSA/Tris-HCl buffer, pH 7·4, overnight. The strips were then incubated with test supernatants, washed four times with 0·05% Tween-20 in PBS and reacted with the peroxidase-labelled anti-mouse immunoglobulins. The peroxidase staining was carried out as described above.

RESULTS

Screening test by ELISA

All supernatants of 331 wells containing growing hybridomas were assayed by ELISA 2–3 weeks after culture. The ELISA study was carried out before cloning of the hybridomas. Culture supernatants of the wells without growing hybridomas were used as controls. A threefold value of the mean optical density of control supernatants at 405 nm was set up as a cut-off point because none of the control supernatants and normal mouse IgG1 showed reactions higher than the cut-off point. Furthermore, none of the supernatants taken randomly from the group below the cut-off point showed a positive reaction against epidermal cells by indirect immunofluorescence.

In the first experiment, four out of 68 (5·9%) supernatants of hybridomas derived from two naive mouse spleens were found to contain antikeratin antibodies with titres greater than the cut-off point (Fig. 1). In the second experiment using a naive mouse, four out of 42 (9·5%) supernatants showed positive reactions. When mice were immunized with non-keratin material such as HTLV-1, ATL cell lysates, and activated human T cells, 12 out of 71 (16·9%), nine out of 54 (16·7%) and 20 out of 96 (20·8%) supernatants, respectively, showed the presence of antikeratin antibodies with titres higher than the cut-off point. When immunized with human epidermal keratins, eight out of 51 (15·7%) supernatants contained antibodies with titres beyond the cut-off point.

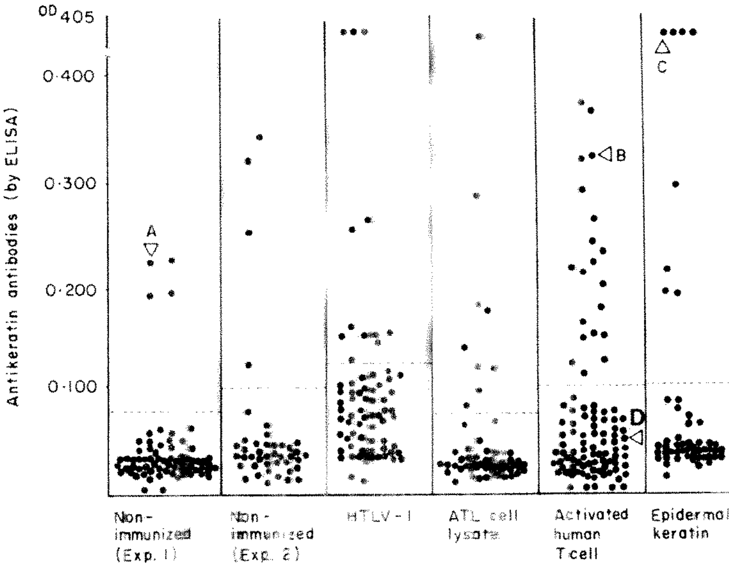

FIGURE 1. Detection of antikeratin antibodies produced by hybridomas derived from naive and immunized mice. Arrow heads A–D correspond to Figure 2a–d.

Immunofluorescence reaction patterns

Supernatants positive for antikeratin antibodies showed various staining patterns on immunofluorescence using human epidermis. Five out of eight supernatants of naive mouse-derived hybridomas reacted with the whole epidermis (Fig. 2a), and one bound mainly to the stratum corneum. The two remaining supernatants did not show any apparent fluorescence on human epidermis.

When mice were immunized with PHA-activated T-cell extracts, seven out of nine positive supernatants tested showed basal cytoplasmic staining in the human epidermis (Fig. 2b, Table 1). One of the supernatants reacted with the whole epidermis. All the antibodies belonged to mouse IgM-class. We could, as a result, obtain a hybridoma clone producing IgM-class antibodies reactive with human basal cells. This antibody demonstrated a positive reaction with human basal cell carcinoma cells, and with the follicular epithelia and 10–20% of the basal cells of BALB/c mouse skin.

Many of the supernatants from hybridomas immunized with keratins or non-keratin materials such as ATL cell lysates and HTLV-I antigens, contained antibodies that reacted with the whole or suprabasal part of the epidermis of human skin (Fig. 2c, Table 1). Among the hybridomas immunized with keratins, we obtained one clone producing IgM-class antibodies against the suprabasal part of human skin as well as in mouse skin. This antibody did not react with human basal cell carcinoma cells.

Cross-reactivity of hybridoma supernatants with vimentin-type intermediate-sized filaments and the exogenous non-keratin immunogens

Because the majority of antikeratin antibodies in the present study were raised in mice immunized with cellular antigens irrelevant to keratins, we checked the possibility that such antibodies were directed against a common epitope(s) shared with intermediate-sized filaments including keratins and the exogenous immunogens.

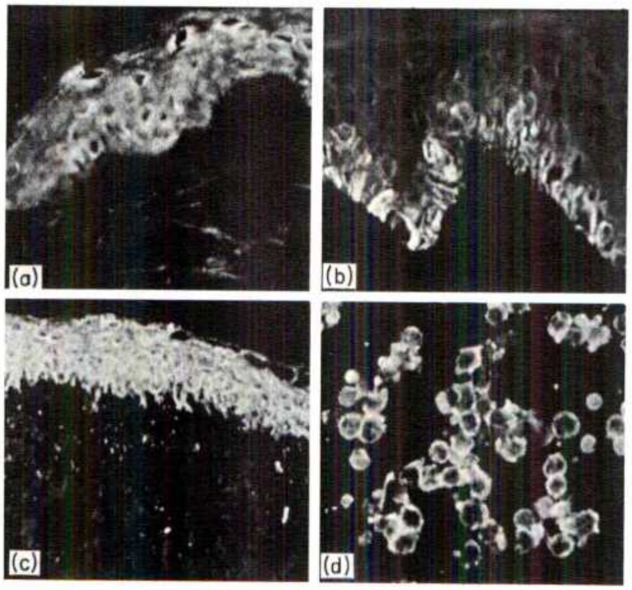

FIGURE 2. Staining patterns against human epidermis and PHA-activated human T cells. Supernatants (A), (B) and (C) in Figure 1 reacted with whole epidermis (a), basal cells (b) and upper epidermis (c), respectively. A supernatant (D) without detectable antikeratin antibodies by ELISA showed positive membrane fluorescence for the activated T cells (d).

TABLE 1. Number of supernatants containing antibodies against keratins and/or original immunogens

Immunogens	Number of supernatants positive for anti keratin antibodies by ELISA (%)	Staining patterns of the antikeratin antibodies in the human epidermis	Number of supernatants containing antibodies to original immunogens (%)	Number of supernatants containing antibodies to both keratins and original immunogens (%)
None (Exp. 1)	4/68 (5·9)	G-cyt: 3/4 SC: 1/4	—	—
None (Exp. 2)	4/42 (9·5)	G-cyt: 2/4	—	—
HTLV-1	12/71 (16·9)	ND	6/71 (8·5)*	0/71 (0)
ATL cell lysate	9/54 (16·7)	G-cyt: 1/3	6/54 (11·1)*	2/54 (3·7)
Activated T-cell lysate	20/96 (20·8)	B-cyt: 7/9 G-cyt: 1/9	16/96 (16·7)†	1/96 (1·0)
Human keratins	8/51 (15·7)	U-cyt: 5/6 B-cyt: 1/6	—	—

G-cyt, general cytoplasmic staining of the human epidermis; SC, stratum corneum; B-cyt, basal cytoplasm; U-cyt, upper layer of the epidermis; ND, not done.

The reactivity of the supernatants against the original immunogens was studied by indirect immunofluorescence using HTLV-1-infected lymphocytes (*), and PHA-activated human T cells (†).

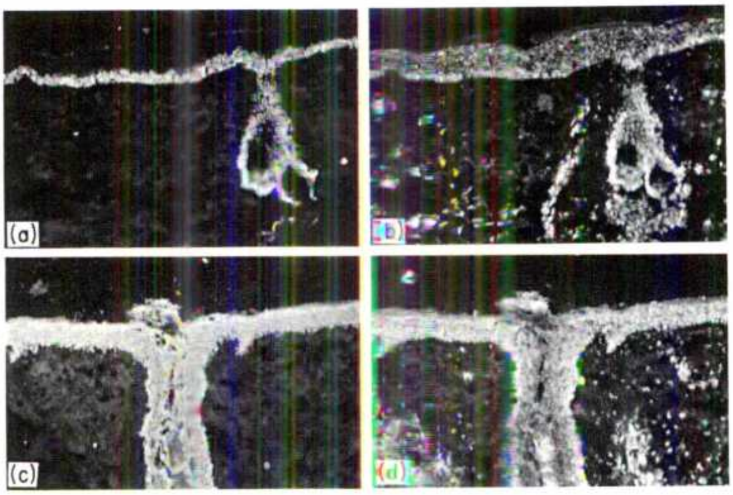

FIGURE 3. Supernatants showing basal cytoplasmic (a) and upper epidermal staining (c). The former was obtained from a mouse immunized with activated human T cells, and the latter from a mouse immunized with human keratins. Ethidium bromide stained sections (b and d) demonstrate that the antibodies of both supernatants do not react with vimentin in fibroblasts and endothelial cells.

Counter nuclear staining with ethidium bromide following indirect immunofluorescence demonstrated that none of the supernatants tested, even though they were obtained from hybridomas immunized with non-keratin antigens such as activated human T cells and ATL cells, reacted with vimentin in the cytoplasm of fibroblasts and endothelial cells (Fig. 3).

When mice were immunized with activated human T cells, none of the supernatants positive for antikeratin antibodies showed cross-reactivity with the original immunogens, i.e. activated human T cells, with one exception that reacted with both keratins and the activated T cells (Table 1). Among the supernatants negative for antikeratin antibodies, about 20% of them showed membrane fluorescence on the activated human T cells. The supernatant corresponding to arrow head (B) in Figure 1, for example showed basal cytoplasmic staining in the epidermis without any reaction against the activated T-cell smear (Fig. 2b). In contrast, the supernatant corresponding to arrow head (D) had no antikeratin antibodies detected by ELISA, but was strongly reactive with the activated human T cells (Fig. 2d).

Similarly, seven out of nine antikeratin antibodies derived from splenic cells immunized with ATL cell lysates did not show any cross-reactivity with the original immunogens by indirect immunofluorescence. When immunized with HTLV-1, the supernatants had specificity to either keratins or ATL cells, but not to both. Six hybridoma clones producing anti-HTLV-1 (p19 or p24) antibodies were eventually obtained among the group negative for antikeratin antibodies (data not shown).

Dot-blot analysis

Reactivity of the supernatants against extracted keratins, a NP40-extract of epidermal sheets, and a cell lysate of PHA-activated T-cells was compared with the reaction patterns of a monoclonal antikeratin antibody, KL-1.

The supernatant corresponding to arrow head (B) in Figure 1, which showed basal cytoplasmic staining (Fig. 2b), reacted weakly with keratins extracted from isolated human keratinocytes and epidermal sheets (Fig. 4). Another supernatant, (C) in Figure 1, showing upper epidermal staining, recognized all keratin extracts obtained from the isolated keratinocytes, epidermal sheets, stratum corneum, and callosities. Neither sample showed any reactivity with the NP40-extract of the epidermis and the activated T-cell lysate. However, a supernatant containing antibodies against activated T cells reacted strongly with the activated T-cell lysate, and weakly with the NP 40-extract of the epidermis. No reaction was observed against keratins (Fig. 4).

Western blot analysis

Selected supernatants were tested by Western blot before limiting dilution. All supernatants with positive antikeratin antibodies, corresponding to arrow heads (A), (B), and (C) in Figure 1, bound to extracted epidermal keratins (Fig. 5). A supernatant (B) containing basal cytoplasmic antibodies reacted with 67–65 and 56 kDa bands of whole epidermal keratins. A supernatant (A) obtained from a naive mouse-derived hybridoma reacted strongly with 67–50 kDa keratins. Another supernatant (C), showing upper epidermal staining, bound to 67–45 kDa keratins and other molecules with large molecular sizes. A monoclonal antibody, KL-1, recognized only 55 (56)–57 (58) kDa keratins in one dimensional gel, as previously reported.[16]

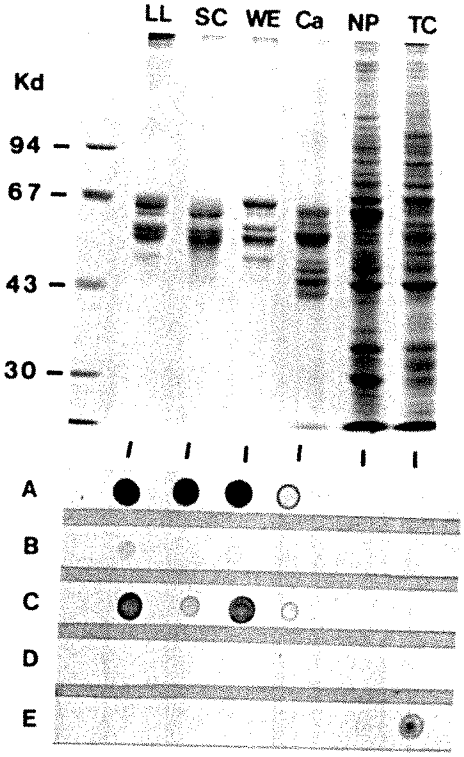

FIGURE 4. Dot-bot analysis and SDS-PAGE patterns of keratins and NP40-soluble fractions. Keratins were prepared from living layers (LL), stratum corneum (SC), whole epidermis (WE), and callosities (Ca). NP and TC indicate NP40-extracts of the epidermis and of PHA-activated human T cells, respectively. Lane A, a supernatant showing upper epidermal staining (C in Fig. 1); lane B, a supernatant showing basal cytoplasmic staining (B in Fig. 1); lane C, a monoclonal antikeratin antibody, KL-1; lane D, control mouse IgG1; lane E, a supernatant reactive with activated human T cells by immunofluorescence.

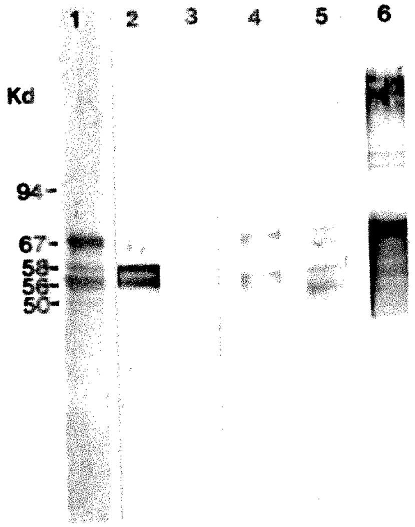

FIGURE 5. Western blot patterns of the supernatants. Lane 1, keratins stained with fast green; lane 2, KL-1; lane 3, control mouse IgG1; lane 4, supernatant showing basal cytoplasmic staining (B in Fig. 1); lane 5, supernatant reactive with whole epidermis obtained from naive mouse-derived hybridomas (A in Fig. 1); lane 6, supernatant showing upper epidermal staining (C in Fig. 1).

DISCUSSION

In the present study, antikeratin antibodies were produced by hybridomas obtained from non-immunized mice and mice immunized with antigens irrelevant to keratins. All the antibodies from naive mice that were tested belonged to the IgM-class. They were autoantibodies but not autospecific because they reacted with mouse and human keratinocytes.

Two hybridoma clones producing an IgM-class antikeratin antibody were previously established from non-immunized mice.[17] Antikeratin antibodies are detected in almost all human sera,[2] and EB virus-transformed B cells can produce various types of antibodies to cytoskeletons including keratins.[9,10] These findings, together with the present study, indicate that B cells exist in normal mice capable of producing antikeratin antibodies.

As compared with the incidence in naive mice, the frequency of supernatants containing antikeratin antibodies was increased 1·5–2 times by immunization of mice with keratins, and about 1·5–3 times in mice immunized with irrelevant immunogens such as human activated T cells, ATL cell lysates, and HTLV-1 antigens. The antibodies produced by the hybridomas demonstrated various immunofluorescence patterns on the epidermis such as staining of the stratum corneum, the cytoplasm of suprabasal cells, whole epidermis, and the cytoplasm of basal cells. These patterns are similar to those of naturally occurring human epidermal cytoplasmic antibodies.[1,2] Some supernatants with low titres of antikeratin antibodies as determined by ELISA did not show positive immunofluorescence to human epidermis (Table 1). This indicates that the ELISA was more sensitive for the detection of antikeratin antibodies than immunofluorescence. Alternatively, the antibodies in the supernatants may have reacted with an epitope that appeared on keratins following treatment with detergents.

The results of dot-blot and Western blot studies also demonstrated that different types of antikeratin antibodies reacted with different keratin subunits. Unlike a previous report,[18] a supernatant that was reactive only with basal cell keratins in human epidermis bound to 67–65 kDa keratin, thought to be a marker for keratinized epithelia, as well as 56 kDa keratin. Furthermore, another supernatant reactive with the upper epidermis bound to the keratin subunits ranging from 67–45 kDa. These results should be interpreted with caution because of the possibility that the supernatants were contaminated with different antikeratin antibodies, and that the antigenic epitopes on keratins were altered by extraction with detergents.

Many of the antikeratin antibodies obtained did not show cross-reactivity with the exogenous immunogens and vimentin-type intermediate filaments. A small number of the supernatants reacted with both keratins and the exogenous immunogens. This test result may reveal the coexistence of different antibodies reactive with each keratin or the original immunogens because the hybridoma supernatants were studied before limiting dilution. The antikeratin antibodies may recognize a common epitope(s) on extracutaneous antigen molecules used for immunization.

The present study indicates that immunization of mice with antigens irrelevant to keratins also increases the production rate of antikeratin antibodies. It is possible that the injection of adjuvant itself causes polyclonal B-cell activation, thereby stimulating antibody production.[19] Souroujon *et al.*[20] have reported that the frequency of autoantibodies to nuclear and cytoskeletal components was much greater in polyclonally activated mouse spleen cells than that of antibodies to exogenous antigens. A high incidence of antikeratin antibodies or epidermal cytoplasmic antibodies has been reported in patients with immunological disorders and following epidermal injury.[1] In these conditions, polyclonal B-cell activation as well as a specific stimulation with keratins released from the damaged epidermis may allow the increased production of autoantibodies against keratins.[21,22] Transformation of B cells may also lead to the production of various types of autoantibodies against cellular components including keratins. Autoantibodies to epidermal components are frequently observed in patients with infectious mononucleosis and myeloma,[1,13] and in virus-infected B cells and human hybridoma cells.[9-12]

Although natural antikeratin antibodies are present in high titres in various disorders their function is uncertain. It has been proposed that natural antikeratin antibodies promote the phagocytosis of leucocytes, thereby removing insoluble keratins.[23] Cohen and Cooke[24] suggested that natural autoantibodies bind to self or self-mimicking epitopes and so prevent the initiation of a damaging autoimmune response.

We conclude that certain B cells capable of producing various kinds of autoantibodies against different keratin subunits exist in normal individuals, and the production of such autoantibodies is increased by both specific antigen stimulation and polyclonal B-cell activation *in vivo*.

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The changing face of dermatology out-patient referrals in the south-east of Scotland

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SUMMARY

A study of out-patient dermatological services (NHS and private) in the south-east of Scotland was carried out by medical staff in the Department of Dermatology in Edinburgh during the month of November 1988. The aim was to assess changes in referral patterns and workload compared with the findings of an identical investigation undertaken in November 1981. Of particular interest were the possible effects of recent publicity campaigns aimed at increasing public awareness about skin cancer.

The medical complement of the dermatology department had changed minimally since 1981 and the population increase in the south-east of Scotland over the same period was 1·5%. During November 1988 1592 new patients and 2037 review patients were seen. This represented an increase of 29·2% and 28·3%, respectively, since 1981. The most striking changes in diagnostic groups were a 173% rise in new cases presenting with benign tumours (excluding viral warts) and a 106% increase in new patients with malignant tumours. Viral warts and eczema were, as in 1981, the second and third most common diagnostic categories amongst new patients. There was a 98% increase in the number of surgical procedures performed on new patients compared with 1981. We conclude that the substantial increase in numbers of both benign and malignant tumours and the consequent doubling in surgical treatments was due to increased public awareness and concern about skin cancer.

Monitoring trends in the number and type of referrals to dermatology out-patient clinics is important in predicting the future developments of our service, although the incidence of skin disease can only be determined accurately by large scale population surveys such as that in Lambeth in 1976.[1] Hospital out-patient referrals will give a guide to changing trends, but may be affected by various factors including the size of the medical establishment, specialist interests, introduction of new treatments for previously refractory disorders, and increased public awareness and concern about skin cancer. Our impression was that recent publicity campaigns about the prevention and early diagnosis of skin cancer had had a considerable effect

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on the number and type of patients seen in clinics. We decided therefore to confirm that impression by repeating a study of the out-patient workload in the south-east of Scotland initially conducted during a 1-month period in November 1981,[2] prior to any publicity campaigns.

METHODS

Dermatology services in south-east Scotland

The population of the study area in the south-east of Scotland in 1988 was 1,189,000, a rise of 18,000 (1·5%) since the previous study in 1981. The distribution of clinical services had altered only slightly through the opening of a new district general hospital in the Borders region.

The major dermatology unit was still located in the Royal Infirmary, Edinburgh where five out of the six consultants were based, the sixth working mainly in Fife. Clinics were held each week in one other Edinburgh hospital as well as at district general hospitals in West Lothian, Fife and the Borders.[2] In-patient facilities were only available in the Royal Infirmary, Edinburgh where there was a 26-bed, 7-day ward and a 15-bed, 5-day unit. Dressing services were limited to the Royal Infirmary, Edinburgh and the Victoria Hospital, Kirkcaldy and the regional phototherapy units were also located at these two hospitals. Each week three private consultation clinics were provided in Edinburgh by two consultants; there were no other private dermatological practices in the region.

Medical personnel

The medical complement within the department had changed minimally since the last study and was responsible for the following NHS half-day sessions (shown in parentheses): one professor (6), five consultants (10,10,10,9,5), one senior registrar (10), one lecturer (10), two registrars (10,10), one associate specialist (2), four clinical assistants (4,6,5,2) and one house physician (10) whose main responsibility was for in-patients. The major expansion of staff had occurred at the clinical assistant level with two additional posts accounting for 11 extra sessions. Of these, five sessions were allocated to cope with the increasing demand for phototherapy and two to conduct pigmented lesion clinics (funded by the Cancer Research Campaign).

All staff participated in the teaching of small groups of undergraduate medical students who were allocated to the department of the Royal Infirmary for 2-week periods for 46 weeks of the year. Most staff also participated in postgraduate teaching, as well as some form of clinical research.

Collection of data

During a 1-month period of November 1988 all members of the dermatology medical staff in the south-east region of Scotland were asked to record the following data on all patients seen in out-patient clinics, as inter-hospital referrals and in private practice:

(a) the total number of new and review patients;
(b) the date of referral and the date of appointment;
(c) the diagnosis;
(d) the number and nature of investigations performed;
(e) the treatment administered.

All data were collected and analysed to give overall statistics for the total number of new and review patients, the waiting time for consultation, the distribution of diagnostic groups and the numbers of surgical or other procedures performed.

Publicity campaigns on skin cancer

Two public education campaigns on the early detection of cutaneous malignant melanoma took place before our 1988 survey. Undoubtedly the 1985–86 programme based in Glasgow,[3] less than 50 miles from Edinburgh, affected the population in our catchment area. Furthermore, Edinburgh was one of seven centres chosen by the Cancer Research Campaign to undertake a similar publicity exercise during the period 1987–89.[4]

RESULTS

Number and nature of consultations

In comparison with 1981 when 1232 patients were seen, 1592 new patients were seen in 1988 (an increase of 29·2%) and 2037 review patients were seen compared with 1587 (an increase of 28·3%). The new: review ratio was 1 : 1·3 concurring exactly with the 1981 study. Diagnoses were recorded in all but 1·8% of the new and 0·7% of review cases. The most common diagnostic groups for new and review patients are shown in Table 1 and are compared with figures for 1981.

It can be seen that the main rise in new patients was predominantly due to an increase in benign and, to a lesser extent, malignant tumours. Of the benign tumour group 30% (197) were pigmented naevi, 26% (173) were seborrhoeic warts and 11% (76) were actinic keratoses. It is of interest to note that in 1981 only 60 patients with pigmented naevi presented to the department over the 1-month study period compared with 197 in 1988. With regard to the malignant tumour group the majority of new patients presented with a basal cell carcinoma. Eight malignant melanomas were diagnosed compared with two in 1981 (Table 2).

Surgical procedures and investigations

Biopsies were performed on 20·6% (328) of new patients and 2·9% (61) of those patients reviewed, a total of 389 in 1 month. This represents an increase of 98% in the number of new patients biopsied compared with 1981.

TABLE 1. Distribution of diagnoses of new and review patients for a 1-month period, 1988 and 1981

	New patients (%) (Total)		Review patients (%) (Total)	
	(1592)	(1232)	(2037)	(1587)
Diagnosis	1988	1981	1988	1981
Benign tumours	39·6	19·3	12·4	6·1
Viral warts	13·5	16·9	22·6	33·0
Dermatitis/eczema	12·8	16·7	15·0	19·0
Malignant tumours	8·3	5·3	9·3	4·1
Psoriasis	4·0	6·7	10·4	10·0
Infections	3·9	7·1	2·0	2·6
Acne/rosacea	3·2	4·4	3·7	4·2
Miscellaneous	12·8	23·6	23·4	21·0

TABLE 2. Distribution of types of malignant tumours amongst new patients seen during a one month period

Diagnosis	1988	1981
Basal cell carcinoma	82	43
Squamous cell carcinoma	9	6
In-situ squamous carcinoma	24	14
Malignant melanoma	8	2

Minor procedures including cryotherapy, curettage and cautery and triamcinolone infiltration were carried out on 21·9% (350) of new patients and 23% (478) of return patients compared with 25·6% (316) and 39·7% (631) of new and review patients, respectively, in 1981. The relative decrease in numbers of minor procedures performed is mainly as a result of a reduction in both new and return patients attending for cryotherapy of viral warts.

Non-surgical investigation was performed in 7% of all patients in 1988 compared with 10% in 1981.

Waiting times

The routine waiting list appointment time in 1988 was 12 weeks compared with 5 weeks in 1981: this might account for the increase in number of early appointments requested, 14% in 1988 compared with 9% in 1981. Requests for urgent appointments (patients seen within 2 days) remained unchanged: 6·7% in 1988 compared with 6·5% in 1981. Of the urgent group, 41% had dermatitis, a further 22% pigmented lesions and the remainder comprised infections, urticaria and vasculitides. Ninety per cent of such referrals were made by the general practitioner and 10% stemmed from other hospital departments.

Distribution of clinical workload

Each consultant conducted an average of four general clinics per week as well as some special interest clinics. Junior staff and clinical assistants conducted an average of four clinics and one minor surgical session each week. Consultant staff saw 49% of new patients and 32% of review patients referred to the National Health Service (NHS) departments. Private practice accounted for only 5·2% of new patients seen during the study month. Of the NHS new patients, 66·5% were seen in Edinburgh hospitals, 18·9% in Fife, 5·4% in West Lothian and 4% in the Borders.

DISCUSSION

Dermatology clinics rank amongst the busiest of the out-patient specialties. In the south-east of Scotland there has been a steady rise in new referrals to our out-patient clinics from 10,800 per annum in 1981 to over 14,000 per annum in 1988.[5] These official statistics do not, however, reflect the reasons for increased referral rates.

Studies of the kind we have performed are useful in assessing changes in patterns of referral, so that adjustments can be made in organisation of clinics and deployment of manpower. We can make a valid comparison of the results of our two surveys as each was conducted during November, so that seasonal fluctuations in referral patterns can be ignored. The population of the south-east of Scotland has also remained relatively stable and there has been only a small increase in staff, mainly of clinical assistants, with a total of 11 new sessions since 1981. The routine waiting list time of 12 weeks in 1988 compared with 5 weeks in 1981 indicates that, in spite of a small increase in staffing, we were unable to keep up with the demand for dermatology out-patient appointments, this increase being 29·2%.

The major difference between the two studies was a rise of 173% and 106%, respectively, in the numbers of benign and malignant tumours seen and the concomitant increase of 98% in the number of biopsies which were performed on new patients. This is a reflection of increased patient anxiety about possible malignant change within benign naevi and also the demand by general practitioners for rapid inspection and removal of pigmented lesions. The impact of media publicity as a stimulus for referral was first described by Rook and Wilkinson in 1979.[6] These anxieties have been fuelled more recently by well-run publicity campaigns promoting the early detection of skin cancer, in particular malignant melanoma.[3,4] These campaigns have contributed to the current inundation of dermatology out-patient departments by patients with pigmented lesions. As a result special pigmented lesion clinics, funded by the Cancer Research Campaign, were established in seven centres throughout the U.K. to provide a rapid screening service which would cater for the increased demand following these publicity campaigns. It is noteworthy that the clinical assistant who ran the Edinburgh pigmented lesion clinic saw 12% of all new patients during the study period. The aim of establishing such special clinics was not only to expedite the referral of patients with malignant melanoma but also to assess whether the publicity campaigns were indeed effective in this respect by recording and comparing the number of thin good prognosis melanomas presenting prior to and following the publicity campaign.

During the study period in 1988, eight malignant melanomas were diagnosed and treated compared with two in 1981. This reflects the increasing incidence of this tumour in Scotland, a rise of 86% during the decade 1979–88 (unpublished data of the Scottish Melanoma Group). However, we (unpublished data) and others in the U.K.[3,4] also have evidence that a larger proportion of thin, good prognosis malignant melanomas are now being removed, presumably as a result of effective publicity campaigns motivating patients to consult doctors sooner.

Any increase in the early detection of malignant melanomas is encouraging but it is disappointing that amongst lesions often referred by general practitioners as possible malignant melanomas there were such a high proportion of seborrhoeic warts (this group alone represented 26% of all the benign tumours seen). It is also apparent that the cosmetic awareness of the public has risen greatly over recent years. Referrals for the removal of benign lesions were often labelled as 'recently changing' or 'recently become inflamed' but, on closer patient questioning, the motive for seeking hospital referral was in fact to have the lesion removed for cosmetic reasons.

One solution to the problem of coping with this exodus from the general practitioner's surgery to the dermatological out-patient department entails the creation of more consultant posts. This is especially pertinent considering that the Government White Paper on the reorganized NHS[7] suggests all patients should be offered a consultation with a consultant rather than with a trainee. In 1981, the proportion of our new patients seen by the consultant staff was 62·5% but by 1988 this figure had fallen to 49%, although actual numbers of new patients seen by consultants had risen by 18%. This is hardly surprising as since 1981 there has been an increase of only five consultant sessions. This still leaves the south-east of Scotland well short of the recommended level of consultant staffing with a figure of 237,000 population per whole time equivalent (WTE); the recommended level of the British Association of Dermatologists being 150,000–200,000 population per WTE. As a result more new patients are being seen by junior staff in training posts and clinical assistants; for those in training this provides wide clinical experience but consultant supervision cannot always be adequately maintained. Furthermore, continuity of care which should be a cornerstone of any out-patient medical service is reduced.

Another solution involves the general practitioner being encouraged to take on some of the bread and butter dermatological tasks such as cryotherapy for warts, skin tags and seborrhoeic warts to relieve congestion in the out-patient clinic. The new General Practice Contract[8] with its promise of remuneration for such procedures, has certainly encouraged many more general practitioners in our area to attend our department for training in these techniques. It seems that this is already having an impact on the numbers of patients attending our clinic for cryotherapy to viral warts.

There are increasing pressures on medical staff to audit every aspect of clinical practice. It is evident that studies such as this, far from being performed every 7 years, will become a regular feature of departmental life. Audit is a double-edged sword having the capacity not only to modify current clinical practice by increasing the efficiency of investigation and management of patients but also by identifying areas of changing needs requiring an increase in or reallocation of expenditure and resources. The continued funding of any department will depend on such assessments of workload, providing evidence of efficient but appropriate management of patients.

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Validation of Sickness Impact Profile and Psoriasis Disability Index in psoriasis

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SUMMARY

A prospective cross-sectional questionnaire study of 32 patients with psoriasis was carried out in order to validate the use of the Sickness Impact Profile (SIP) in psoriasis and compare its sensitivity with the Psoriasis Disability Index (PDI). Overall PDI scores, but not overall SIP scores, correlated well with PASI scores ($P < 0.05$). There was good correlation between the PDI and overall SIP scores ($P < 0.01$). Psychosocial factors are more severely impaired than physical activities in patients with psoriasis.

It is now possible to directly compare the disability experienced by psoriatic patients with that experienced by patients suffering from other systemic diseases, using the SIP. The PDI is an appropriate method to give a rapid overall measure of psoriasis disability.

Methods of disease assessment are needed in dermatology in the clinic to aid routine management and in clinical research to assess the value of new management techniques. Many methods are used to measure the clinical severity of psoriasis,[1] but these do not assess the influence of the disease on the patient's life. An ability to measure this effect is needed to convincingly demonstrate the importance of skin disease and to argue effectively for resources for dermatology. There are methods to assess the impact on patients of psoriasis[2] and of acne,[3] but these questionnaire techniques are disease-specific and inappropriate for comparing one disease to another. A method is needed which has been validated in non-dermatological diseases in order to make comparisons of the disability experienced by patients with skin disease or, for example, angina. The Sickness Impact Profile (SIP) is a detailed general measure of the impact of disease on patients' functional behaviour which has been used in hypertension, angina, and in the assessment of the side-effects of different new systemic drugs.[4] However, the SIP has not previously been used to assess disability in skin disease.

The purpose of this study has been to validate the use of the SIP in psoriasis and to compare its sensitivity with the Psoriasis Disability Index (PDI), thereby allowing the disability

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experienced by patients with psoriasis to be compared to that experienced by patients with non-dermatological problems.

METHODS

Ethical permission for this study was given by the Joint Medical Ethical Committee of the University Hospital of Wales. Thirty-two patients (15 male, 17 female) with psoriasis were recruited sequentially to this study. The patients were aged from 14 to 73 years (median 35 years); 23 (72%) were in-patients admitted because of their psoriasis. Nine patients were attending the dermatology out-patient clinic. The nature of the study was described to each patient by A.Y.F.; no patient declined to take part. The PDI and the SIP questionnaires were explained to each patient by M.S.S. in a standard scripted fashion and then completed by each patient with M.S.S. present. A measure of the extent and severity of the patient's psoriasis was recorded by G.K.K. using the PASI method.[5]

The results were analysed using Spearman rank correlation coefficients, the Mann–Whitney U-test or the K–W one-way ANOVA test, as appropriate.

Questionnaire details

The Psoriasis Disability Index. This is a short compact questionnaire which in this study consisted of 15 questions answered on a 1 to 7 linear analogue scale representing grades from 'not at all' to 'very much'. The 15 questions were grouped under five headings: daily activities (five items), work or school (three items), personal relationships (two items), leisure (four items) and treatment (one item). This version of the PDI questionnaire therefore consisted of a more representative selection of questions than that described previously.[2] When completing the PDI, patients were asked to consider the effect of the disease on them over the previous month only. The PDI score is calculated by summing the scores for each question.

The Sickness Impact Profile. This is a detailed performance-based questionnaire consisting of 136 health-related statements in 12 areas of daily activity which require tick-box answers. Three of these areas (body care and movement, mobility and ambulation) concern a physical dimension (45 questions), while four others (emotional behaviour, social interaction, alertness behaviour and communication) concern a psychosocial dimension (48 questions). Five other independent categories cover sleep and rest (seven questions), home management (10 questions), work (nine questions), recreation and pastime (eight questions), and eating (nine questions). For the purpose of completing the SIP, the patients were asked to consider the effects of the disease on them that day, in contrast to the previous month for the PDI. In addition to the detailed statement questions, patients were asked to give an 'overall health' subjective statement, choosing from a range of 'very good' to 'very poor'.

The SIP is self-administered and is found to be free of ambiguity, taking 15–20 min to complete. The SIP score is calculated by summing the scale values (weightings based on estimates of the relative severity of each dysfunction) of items ticked divided by the sum of the scale values for all items multiplied by 100. Thus, the SIP per cent score could be easily calculated for the overall SIP questionnaire as well as for each category and dimension.[6]

In administering such a questionnaire it would be expected that 'normal' control subjects would give positive answers to none or only a very few of the statement questions posed. This has been confirmed by Salek and VandenBurg[4] and to indicate the order of magnitude of positive results that might be expected from a control population, values from that study of 50 healthy volunteers are given in Table 1.

TABLE 1. Functional impairment in patients with psoriasis as measured by the Sickness Impact Profile (SIP): SIP scores for psoriatic patients and for healthy volunteers[4]

Category or dimension	Number of items	Mean (SD) scores psoriasis patients ($n = 32$)	Mean (SD) scores healthy adults ($n = 50$)
Overall SIP	136	9·9 (8·7)	0·57 (1·7)
Physical dimension	45	3·6 (7·0)	0·37 (1·7)
Body care and movement	23	3·4 (5·6)	0·28 (1·4)
Mobility	10	4·0 (8·9)	0·20 (1·4)
Ambulation	12	3·9 (11·3)	0·70 (3·6)
Psychosocial dimension	48	12·6 (12·4)	0·50 (2·1)
Emotional behaviour	9	18·9 (19·2)	0·92 (3·8)
Social interaction	20	15·9 (16·3)	0·19 (0·9)
Alertness behaviour	10	8·4 (19·0)	0·96 (6·8)
Communication	9	4·3 (8·8)	0·19 (1·3)
Independent categories			
Sleep and rest	7	15·2 (12·9)	0·25 (1·7)
Home management	10	12·0 (16·6)	1·05 (6·4)
Work	9	27·1 (31·0)	0·65 (2·6)
Recreation and pastime	8	20·6 (20·9)	1·00 (3·6)
Eating	9	2·9 (5·3)	0·00 (0·0)

The Psoriasis Area and Severity Index. In the PASI scoring system,[5] four main body areas are assessed: the head, trunk, upper extremities and lower extremities, corresponding to 10%, 20%, 30% and 40% of the body area, respectively. The area of psoriatic involvement of these four main areas is given a numerical value from 0 to 6 corresponding to 0–100% involvement. In each area erythema, infiltration and desquamation are assessed on a 0–4 scale. The PASI score (0–72) is then calculated to give an overall score for clinical degree of psoriasis.

RESULTS

The clinical severity of psoriasis in the patients studied ranged from PASI scores of 2–24, median 5·5. The scores for the PDI ranged from 7 to 88, median 38, with the following distribution: PDI < 31 (10 patients) PDI $= 31$–50 (11 patients) and PDI > 50 (11 patients). The PDI scores correlated with the PASI scores ($rs = 0{\cdot}40$, $P < 0{\cdot}05$). In contrast, overall SIP scores did not correlate with PASI; this may be accounted for by the differences in the type of questions asked in the two questionnaires.

There was good correlation between the PDI and the overall SIP score ($P < 0{\cdot}01$) and strong correlations between the PDI and all SIP subsections, except with bodycare, alertness, communication and eating (Fig. 1). The scores for the SIP are given in Table 1, along with scores from a healthy group of volunteers with a similar age and sex distribution for simple comparative purposes.[4] The overall SIP mean score for the patients with psoriasis was 9·9 ($SD = \pm 8{\cdot}7$, $n = 32$). As expected psoriasis had only a moderate impact on the patients' ability to move around or take care of themselves, but the psychosocial effects of the disease were profound. There was also a major impact on the patients' ability to sleep, manage their homes,

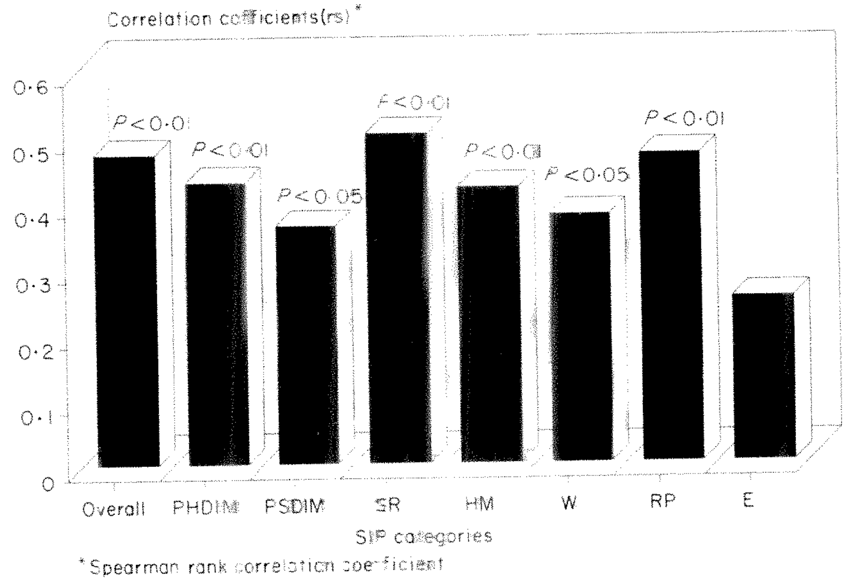

FIGURE 1. Graph to demonstrate correlation between PDI and overall SIP scores. Overall, overall SIP score; PHDIM, physical dimension; PSDIM, psychosocial dimension; SR, sleep and rest; HM, home management; W, work; RP, recreation and pastime; E, eating.

and on their recreational activities. Work was an area particularly affected, reflecting substantial numbers of patients who are unemployed due to their psoriasis (32%).

When the results for the 15 male and 17 female patients were compared, there were no significant differences in PASI, PDI, overall SIP scores or in the SIP subsections. These findings were further confirmed by the lack of correlation with gender in all areas examined as demonstrated by Spearman's rank correlation coefficients. Similarly, age did not correlate with PASI, PDI or SIP, except for the subsections of home management, ambulation and employment ($rs > 0{\cdot}33$). The overall SIP score and most SIP subsections correlated well ($P < 0{\cdot}01$) with the patients' global-rated health assessment (i.e. very good, good, fair, poor, very poor). The patients' global rating of overall health correlated most closely with psychosocial behaviour ($rs > 0{\cdot}46$). The PASI score did not correlate with patients' global rating of overall health. However, those with only a moderate area of psoriasis involvement did have a better perception of their overall health.

DISCUSSION

This study has demonstrated that the SIP may be effectively used in patients with a skin disease such as psoriasis. It has also demonstrated that the PDI, a much shorter and more compact disease-orientated measure, is effective in measuring the same disability. As the two questionnaire methods are conceptually quite different, one arising from interviews with patients with a specific disease, while the other is designed as a general measure of health status applicable to chronic and acute illness, the close correlation between their final scores indicates that they are both successful in measuring the same 'core' of the patients' problems. These techniques may also provide a useful measure for monitoring drug therapy in situations such as psoriasis where functional results rather than laboratory parameters or survival are of primary interest. Thus instruments such as the SIP and PDI, if used together, could be complementary in identifying the breadth and extent of a patient's physical and psychosocial functional limitations. Our findings have shown that chronological age and sex do not have any bearing on psoriatic patients' perception of their health status. However, age may affect the degree of a patient's dependency in daily living with respect to various support services. Unemployment is certainly an important issue among patients with psoriasis and thus return to work constitutes one of the major aims of treatment.

It is now possible to compare quantitatively for the first time the disability experienced by psoriatic patients with that experienced by patients suffering from other systemic diseases. For example, data is available concerning cardiovascular disease.[7] The mean SIP score for patients with hypertension was 7·0 (SD = $\pm 7{\cdot}7$, $n = 52$), with angina the mean was 8·2 ($\pm 7{\cdot}4$, $n = 50$) while with cardiac failure the mean was 10·4 ($\pm 8{\cdot}1$, $n = 53$). In the current study the mean score for patients with psoriasis was 9·9 (SD = $\pm 8{\cdot}7$, $n = 32$). The interviewer was the same in each study, thereby removing one possible source of bias. Although the psoriasis patients were not directly matched for age with the patients in the other study, the comparison indicates that a group of hospital-treated patients with psoriasis suffer less disability than patients with cardiac failure but may suffer at least a level of disability equivalent to that of patients with angina or hypertension.

As part of the development of the SIP, comprehensive testing has previously been carried out to investigate its test–retest reliability.[6] The correlation coefficients of individual SIP items averaged 0·5, while for the 12 category scores the average was 0·82; this indicates good reproducibility for individual items and excellent reproducibility for category scores. The reliability of overall scores did not vary by type or by level of sickness. In our reported study reproducibility when used in a dermatology context was not studied, though this is now under investigation.

We have previously demonstrated that the PDI may be used to measure the benefit to the patient of in-patient topical treatment of psoriasis.[2] Clearly the SIP may potentially be used in a similar way to assess the benefit of different management approaches. The 136 questions of the self-administered SIP take only about 15–20 minutes to complete. The SIP was designed to be used in a very wide variety of disease states and there is a 'core' of SIP questions which are repeatedly of relevance to patients with skin disease, and many which are consistently irrelevant. It may therefore in theory be possible to condense the SIP for use in the dermatology setting, but in so doing it would lose its major advantages of giving breadth of information and being applicable between different disease states.

In research studies or in the clinical situation where a simple rapid overall measure of disability is required either in individuals or in groups of patients with psoriasis, it is appropriate to use the PDI. However, in situations where it is necessary to compare disability in psoriasis or other skin diseases with non-dermatological disease, it is appropriate to use the SIP. The SIP also provides a more comprehensive picture of the impact of disease on a patient's life style. Data provided here should encourage acceptance by physicians and awareness of the need for such measures in both clinical practice and research.

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Pigmented spindle cell naevus

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SUMMARY

We report 22 cases of pigmented spindle cell naevus (PSCN). The usual appearance of these naevi is that of a heavily pigmented papule found mostly on the legs of young patients. Histologically, PSCN was characterized by symmetrical proliferation of spindle-shaped pigmented melanocytes grouped in large junctional nests. Pagetoid spread of single cells in the overlying epidermis was frequently found. In our opinion, PSCN is a distinctive benign acquired melanocytic naevus that in the past has been frequently misdiagnosed as atypical Spitz naevi or malignant melanoma.

Pigmented spindle cell naevus (PSCN) was first described in 1975 by Reed *et al.*[1] This was considered as a distinct variant of the Spitz naevus. The usual clinical appearance of these lesions was of a slightly dome-shaped smooth and heavily pigmented papule. These were generally 3–6 mm in diameter and have a sharp demarcation from the surrounding skin. They mostly occur on the legs of young women. Histologically they are characterized by symmetrical proliferation of spindle-shaped pigmented melanocytes grouped in large nests confined to the dermo-epidermal junction and papillary dermis.

So far there have been a number of reports in the literature of PSCN[2–9] and recently variants have been described.[10] It is likely that the condition is not rare and that many cases are misdiagnosed either as malignant melanoma or as Spitz naevi.

The purpose of this report is to present the clinical and histopathological findings of 22 cases of PSCN from our institution. In our opinion, this condition can be differentiated from Spitz naevi and from malignant melanoma.

METHODS

The cases reported were from the files of the dermatopathology laboratory of our hospital from 1984 to 1989. The clinical details were obtained from either the available notes or from the referring pathologists.

At least four sections stained with haematoxylin and eosin were examined for each case and in

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several, multiple serial sections were performed on the lesions. The histological findings were evaluated separately by each author and there was good concordance in the scoring.

RESULTS

There was a total of 22 lesions from 22 patients (14 female, eight male). The age range of the patients was 5–34 years (mean 16 years). Sixteen of the lesions were on the legs (including buttocks) and six on the arms (including the shoulders) (Fig. 1) . In our patients none of the lesions were on the head, neck or trunk.

The papules had a characteristic clinical appearance, they were small and varied in diameter from 1 to 10 mm (mean 4 mm). They were heavily pigmented, slightly dome-shaped tumours that were sharply demarcated from the adjacent normal skin and had a smooth surface (Fig. 2). The history prior to excision varied from 4 months to 17 years (mean 6 years). None of the lesions were described as being present at birth. The suggested clinical diagnosis included malignant melanoma (three cases), dysplastic naevus (eight cases), melanocytic naevus (eight cases), angiokeratoma circumscriptum (one case) and PSCN (two cases).

Histologically, 19 of the 22 lesions (86%) were junctional naevi and three (14%) were compound naevi. No entirely intradermal naevi were seen. The lateral demarcation was sharp at the margins in all of the lesions. Usually there were well-defined nests of junctional spindle cells (Fig. 3). Twenty of the lesions showed on low power fairly good symmetry, whereas two of the lesions were composed of junctional nests in half of the tumour and isolated spindle cells at the dermo-epidermal junction in the other half. Junctional lentiginous melanocytic hyperplasia was seen in eight of the lesions. The predominant morphology of the cells in all the lesions was spindle-shaped (Fig. 4). Various locations and shapes of nests of naevus cells were found, but the 'round nests' of spindle cells at the bases of the elongated rete ridges were most frequently seen. Occasional bridging was seen in two of the cases. Cytological atypia was not seen and normal

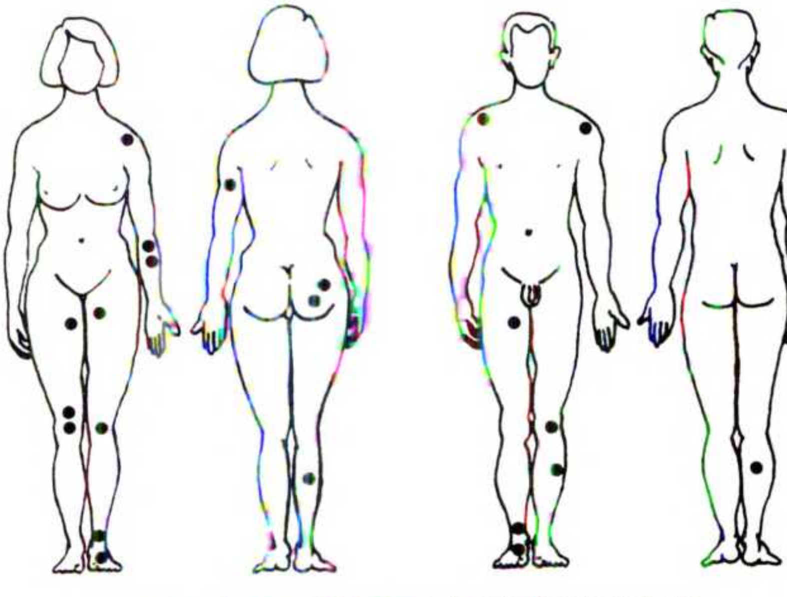

FIGURE 1. Lesions of PSCN located predominantly on the leg.

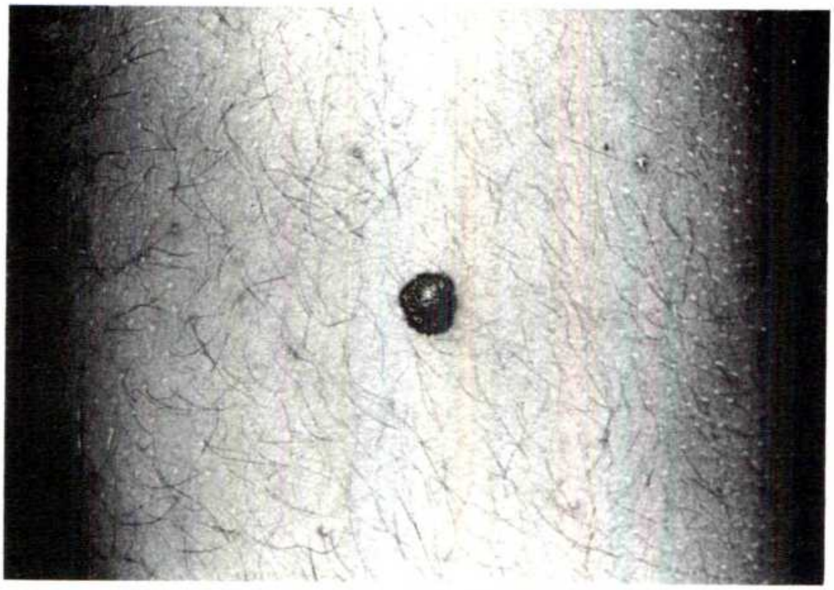

FIGURE 2. Pigmented lesion on the thigh of a young male.

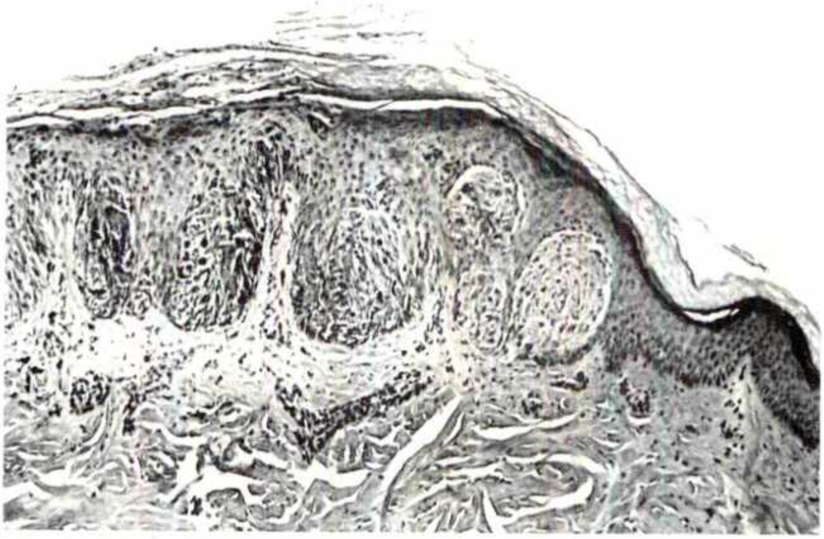

FIGURE 3. Sharp lateral demarcation that was present in all lesions.

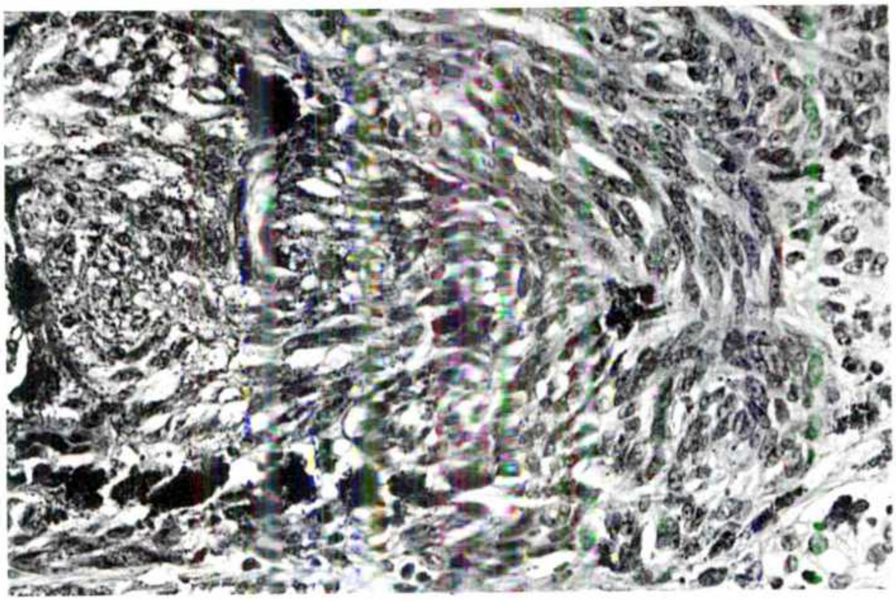

FIGURE 4. Spindle cells of the lesions showing abundant melanin but no atypia.

mitotic figures were seen in only one of the lesions. In the three compound naevi there was a decrease in cell size and the amount of cytoplasm at the base of the lesion. This maturation at the base was evident from the cell size but it remained spindle-shaped.

Epidermal invasion by single cells or by small nests was seen in 19 of the cases. This intraepidermal spread was always in the overlying epidermis of the naevus, and there were no solitary melanocytes or nests extending laterally beyond the main component of the tumour. Junctional nests of spindle melanocytic cells were seen in the upper part of the intradermal eccrine duct (Fig. 5) and in the infundibular segment of the hair follicles in the tumour in seven of the lesions. Eosinophilic globules (Kamino bodies) were found in seven lesions at or near the dermo-epidermal junction and in one case these were heavily pigmented. Some degree of epidermal hyperplasia was identified in 14 of the lesions, being moderate in 10 and marked in four. In three of the cases there was a rather atrophic epidermis.

Nearly all (21 out of 22) had easily identifiable pigment both in the nests of tumour cells and in the overlying epidermis. In the most pigmented lesions there was an abundant amount of melanin in the horny layer and many heavily pigmented macrophages in a band-like arrangement beneath the tumour nests. Varying degrees of inflammatory responses were seen at the base of the lesion in all but one of the cases. These were most marked in six and moderate in 15. A slight lamellar fibroplasia of the papillary dermis was identified in only one of the cases. Some degree of capillary proliferation in the superficial vascular plexus was seen in all but two of the lesions.

DISCUSSION

Pigmented spindle cell naevus is a clinicopathological entity that is under-recognized by dermatologists and dermatopathologists. In a series of cases of PSCN over a period of 5 years[3]

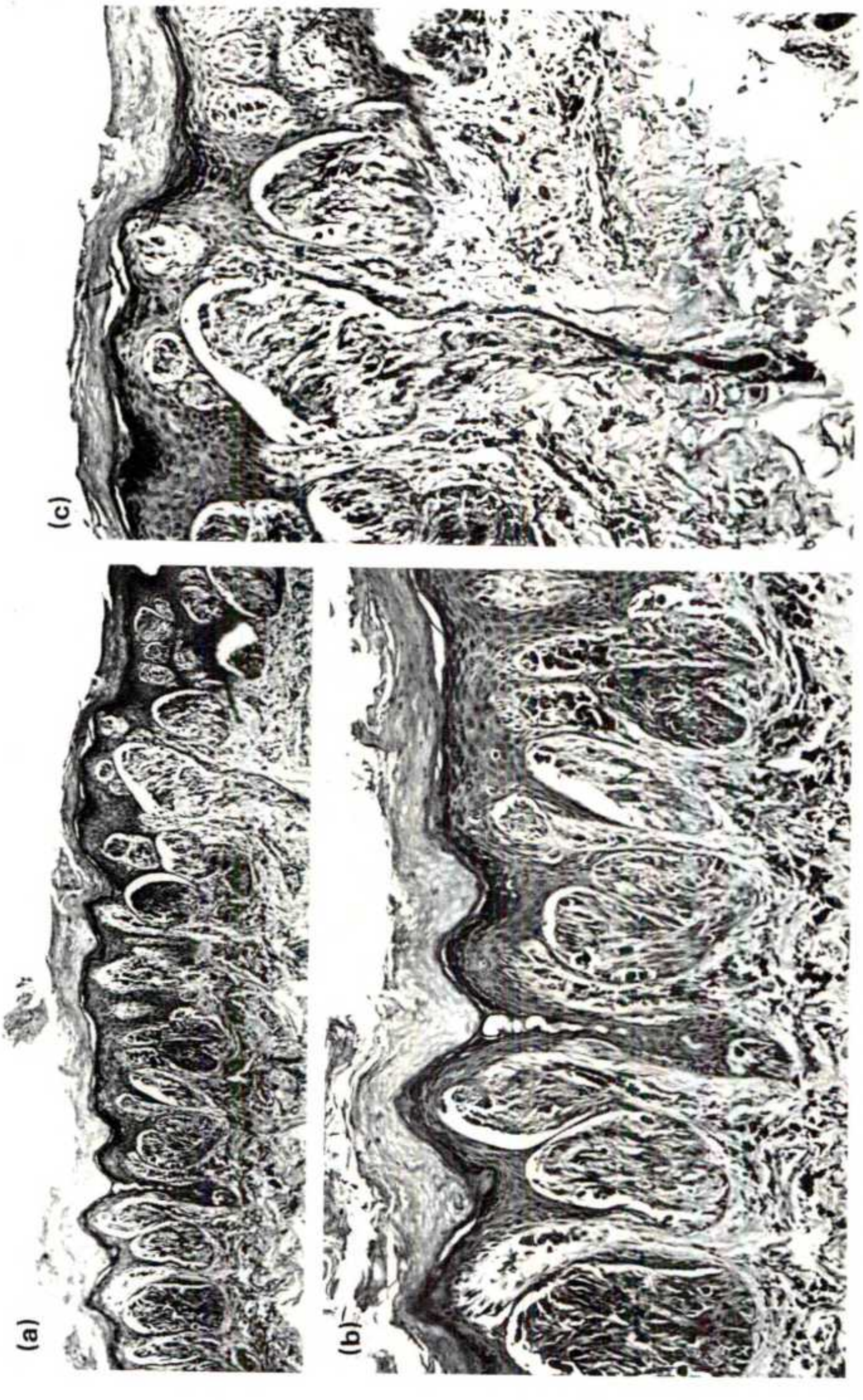

FIGURE 5. (a) Typical PSCN composed of junctional nests of spindle cells. (b) Close-up view of spindle cells. (c) Junctional nests of spindle cells in the upper part of an intradermal eccrine duct.

TABLE 1. Cases of pigmented spindle cell naevus reported in the literature

Author	Year	Number of cases	Sex of the patients male/female	Age (years)		Site of the lesions		
				Age range	Average age	Lower limbs	Upper limbs	Other areas
Gartmann[4]	1981	28	9/19	4–40	?	9	7	12
Sagebiel *et al.*[2]	1984	91	30/60	2·5–56	25·3	38	23	30
Sau *et al.*[9]	1984	95	39/56	88 cases in the first four decades of the life		75%		25%
Schubert[6]	1986	8	3/5	3–50	?	3	0	5
Smith[3]	1987	40	14/26	2–44	22·8	22	4	14
Kolde *et al.*[7]	1987	2	1/1	6–24	15	2	0	0
Barnhill *et al.*[10]	1989	7	4/3	6–39	29	4	3	0
This series		22	8/14	5–34	16	16	6	0

there were only four cases in which the correct diagnosis was previously suggested by the clinicians. In our series of patients the correct diagnosis was only suspected in two of the 22 cases. However, in all the published reports on PSCN there is a marked similarity in the pathological and clinical features of the cases (Table 1). The typical lesion is a small pigmented naevus most frequently located on the legs of young females. Because of the marked pigmentation of the lesion there is often a suspicion that it is a malignant melanoma. The presence of large pigmented spindle cells on histology may frequently lead to the diagnosis of malignant melanoma by the pathologist, who is not aware of this variant of benign melanocytic naevus. The histological features are quite characteristic and PSCN should be differentiated clearly from malignant melanoma and Spitz naevus. Recently, a variant of PSCN has been reported with features of Spitz naevus[10] in which there were fascicles of plump or slightly epithelioid spindle cells with the presence of cytological atypia. It is our opinion that lesions such as this should be classified as cases of Spitz naevus with abundant melanin pigmentation.

A frequent finding in our series of cases was the pagetoid spread of single cells in the overlying epidermis. This feature in a pigmented melanocytic lesion raises the possibility of a malignant melanoma of the superficial spreading type.[11] Intraepidermal spread of tumour cells in PSCN is almost always confined to the overlying epidermis with no solitary melanocytes or nests of atypical melanocytes extending laterally beyond the main component of the naevus. In malignant melanoma this pagetoid spread usually extends beyond the lateral margins of the lesion.

There is, as yet, little information as to the biological behaviour of PSCN, but so far in the documented cases there have been no reports of metastases.[2,3] This condition should be regarded as a benign melanocytic naevus.

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Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare

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SUMMARY

Using an antibody to S100 protein, the number of dendritic cells above the basal layer in the epidermis was assessed in necrobiosis lipoidica and granuloma annulare. A statistically significantly higher number of these cells was found within the epidermis in necrobiosis lipoidica compared with granuloma annulare and normal skin. The numbers were similar to those seen in sarcoidosis and tuberculous reactions in the skin, which raises the possibility of an immune pathogenesis for necrobiosis lipoidica.

Although the clinical appearances of granuloma annulare and necrobiosis lipoidica are quite distinct,[1] histologically the two conditions have similar features. Both show a variable degree of collagen degeneration within the dermis, with an associated histiocytic inflammatory reaction. This reaction takes the form of a palisade of histiocytes, some of which may be epithelioid, with occasional multinucleate giant cells.[2,3] Langerhans cells form a population of dendritic cells that are widely distributed throughout the skin, in the stratified squamous mucosal epithelia, lymph nodes and lymphatic vessels draining the skin. They are found in the largest numbers, however, within the epidermis where they function as antigen-presenting cells. Their density within the epidermis is similar in most body sites, except for the palms and soles where the numbers of cells are significantly lower.[4]

Langerhans cells were first identified by Paul Langerhans, in 1868, using a gold-chloride technique. They may also be demonstrated using a variety of methods including membrane ATPase, monoclonal antibody to the T6 antigen, and ultrastructurally by the presence of Birbeck granules. A subset of these cells can be identified using an antibody to S100 protein.[5] This subset of dendritic S100 protein positive cells within the epidermis has been counted in biopsies from patients with necrobiosis lipoidica and granuloma annulare.

METHODS

Fifteen biopsies were taken, and of these seven were of necrobiosis lipoidica and eight granuloma annulare. The tissues were formalin fixed and paraffin embedded. Staining with

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antibody to S100 protein was performed using a standard peroxidase antiperoxidase technique. The sections were trypsinized before application of primary antibody (D.A.K.O. Rabbit anti-cow S100 Code Z311 lot 026) at a final dilution of 1:100. Control sections of normal skin adjacent to non-neoplastic/non-inflammatory lesions, and of normal skin removed at autopsy (20 cases) were used. The numbers of basal cells overlying the intradermal lesion were counted (500–1000 cells), and the number of epidermal dendritic S100 positive cells lying above them were counted. Using this method, S100 positive, basal melanocytes were excluded. Counts were expressed as the number of epidermal dendritic S100 positive cells per 200 basal cells.

Student's unpaired *t*-test was used to assess statistical differences between necrobiosis lipoidica, granuloma annulare, and the control group.

RESULTS

All the biopsies were of predominantly lesional skin. There was no disparity between the histological and clinical diagnoses in any of the cases. All showed the recognized features of collagen degeneration, some having palisaded granulomas. In the majority, the full thickness of the biopsy that included the lower reticular dermis was involved by the lesions.

Dendritic cells were seen positively stained lying between keratinocytes, within the epidermis (Fig. 1). There was an increase in numbers of epidermal dendritic S100 positive cells in both granuloma annulare (mean 8·0) and necrobiosis lipoidica (mean 24·5), compared with normal skin controls (mean 3·7) (see Table 1). The numbers were higher in necrobiosis lipoidica than granuloma annulare ($0{\cdot}001 > P > 0{\cdot}0005$); but there was no statistical difference between granuloma annulare and the control group ($0{\cdot}15 > P > 0{\cdot}10$).

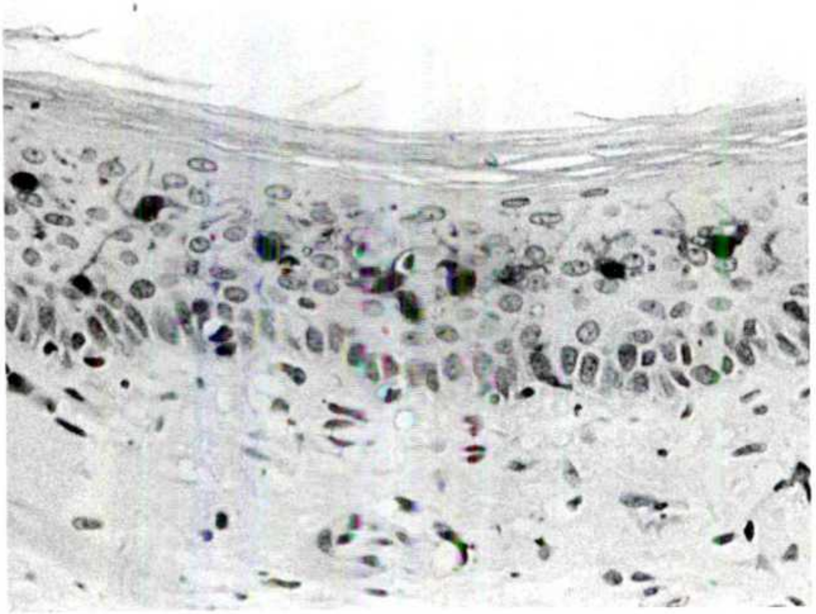

FIGURE 1. Dendritic S100 protein positive cells seen within the epidermis in a case of necrobiosis lipoidica (× 204).

TABLE 1. Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare

	Dendritic S100 positive cells per 200 basal cells		
	Granuloma annulare $n=8$	Necrobiosis lipoidica $n=7$	Normal skin $n=20$
	4·8	17.9	—
	8·8	30·7	—
	11·9	22·0	—
	9·2	20·8	—
	4·9	33·2	—
	6·3	20·4	—
	13·2	26·2	—
	5·1		
Mean	8·0	24·5	3·7
Range	4·8–13·2	17·9–33·2	1·6–5·8
SD	3·3	5·7	1·2

Of the seven patients with necrobiosis lipoidica one had diabetes mellitus and the number of S100 positive dendritic cells within this patient's biopsy was in the same range as the remaining six. None of the eight patients with granuloma annulare were known to be diabetic.

DISCUSSION

Although clinically different, the similarity of the histological features of granuloma annulare and necrobiosis lipoidica raises the possibility of a common pathogenesis. We have determined the dendritic S100 protein positive subset of the Langerhans cell population in the epidermis in these two conditions. There was a significantly higher number of S100 positive dendritic cells within the epidermis, above the basal layer, in necrobiosis lipoidica compared with both normal skin and granuloma annulare. In the latter the numbers of cells were elevated, but not to a significant level above normal. This suggests that the pathogenesis of the two conditions may be different. We have found that the numbers of S100 positive epidermal dendritic cells are elevated to a similar level to that seen in necrobiosis lipoidica in sarcoidosis, positive Kveim test biopsies, tuberculous leprosy and other tuberculous reactions (unpublished data). A cell-mediated, type IV delayed-hypersensitivity reaction is thought to be common to all these conditions. The increased numbers of S100 and dendritic cells within the epidermis in necrobiosis lipoidica may be a reflection of a similar pathological process.

The widely accepted view is that necrobiosis lipoidica is a result of collagen degeneration with a subsequent histiocytic inflammatory reaction.[2] However, the initiating stimulus to degeneration remains unknown. An immune complex vasculitis has also been proposed; direct immunofluorescence demonstration of IgM and C3 in vessel walls, and fibrinogen within necrobiotic areas, lends some support to this theory;[6,7] however, other workers have found these to be present only rarely.[8] There is also little histological evidence of a vasculitis, the vessels showing only a mild degree of intimal thickening. The association of necrobiosis lipoidica with diabetes mellitus has led to the suggestion that the changes may be a result of diabetic microangiopathy.[9] Vascular changes, however, are often absent, and the affected vessels are usually larger than those involved in diabetic microangiopathy. Only one of our cases had diabetes mellitus.

In granuloma annulare the primary event is also thought to be collagen degeneration. Electron microscopy shows degeneration of elastic and collagen fibres in necrobiotic areas.[10,11] Vascular changes are few.[10] A cell-mediated immune reaction has been implicated with the demonstration of increased numbers of T helper/inducer lymphocytes within the granulomatous infiltrate, an increased number of OKT6 positive Langerhans cells within epidermis and dermis, and increased HLA-DR antigen and interleukin 2 receptor expression.[12] The increased numbers of S100 positive cells in granuloma annulare lends some support to these findings, but the numbers are higher in necrobiosis lipoidica and other known type IV delayed hypersensitivity reactions.[5] In conclusion, an increase in the number of epidermal dendritic S100 positive cells above the basal layer, as well as implicating a cell mediated immune-reaction, may provide an adjunct to histological means of distinguishing between necrobiosis lipoidica and granuloma annulare.

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Anxiety and depression in patients with chronic urticaria and generalized pruritus

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SUMMARY

Thirty-four dermatology out-patients with chronic idiopathic urticaria and 34 with idiopathic generalized pruritus were investigated using standardized self-assessment psychological questionnaires to determine the incidence of significant symptoms of depression and anxiety. These patients were compared with age- and sex-matched but otherwise unselected general dermatology out-patients. Using the Beck depression inventory, significantly more patients with generalized pruritus (32·4%) had depressive symptomatology (score > 14) than controls (13·2%, $P < 0.05$). Although more patients with chronic urticaria had depressive symptomatology (14·7%) than controls (4·4%), the difference was not statistically significant. Using the Speilberger state-trait anxiety inventory there were no significant differences between the patients with pruritus or urticaria and their controls with respect to state or trait anxiety scores above the upper 90% probability limit for the general population. Thus, significant depression may be expected in a substantial proportion of patients with idiopathic generalized pruritus but in a relatively small proportion of those with chronic urticaria.

Psychological factors have been widely considered to be important in patients with chronic urticaria and idiopathic generalized pruritus. Most reports have concentrated on psychodynamic theories of causation of these disorders. However, of more practical importance to clinicians is whether psychological problems are a significant feature of patients with these disorders. Several studies have suggested that the incidence of clinically significant depression and anxiety in patients with chronic urticaria is high, but these studies have incorporated selected patient groups.[1-3] Patients with idiopathic generalized pruritus are often considered to have psychogenic pruritus. A tendency towards anxiety and, in particular, depression are psychological characteristics which have been commonly ascribed to these patients.[4,5] However, evidence from controlled studies to support these views has been lacking.

We have determined the incidence of significant depression and anxiety in dermatology out-patients with idiopathic chronic urticaria and generalized pruritus.

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PATIENTS AND METHODS

The study comprised 34 patients with chronic idiopathic urticaria and 34 with idiopathic generalized pruritus who were all new patients attending a dermatology clinic (Table 1). Patients were included if their symptoms had been present for at least 3 months and if no specific cause could be identified by detailed history, examination and appropriate investigations. Patients with predominantly physical urticaria were excluded. The patients were compared with age- and sex-matched, but otherwise unselected, new dermatology out-patients in a ratio of 1:2.

Each patient was asked to complete two questionnaires. The Beck depression inventory[6] (BDI) is a 21-item symptom questionnaire scoring 0–63 which is of proven value in screening for depression in the medically ill.[7,8] A score of 14 or above is generally considered to indicate depressive symptomatology and a score of 21 or above to indicate severe or clinical depression. The Speilberger state–trait anxiety inventory (STAI) is an anxiety symptom questionnaire which has two components each with 20 items scoring 20–80. State anxiety refers to an emotional state at the time of investigation and may be affected by stressful situations such as out-patient attendances. Trait anxiety refers to proneness to anxiety and is a more stable measure. The STAI has been widely used and norms are available for the general population.[9] There is no accepted cut-off score which indicates significant anxiety. We chose scores above the upper 90% probability limit (1·645 SD above the mean) for the general adult population[9] to indicate significant anxiety.

These questionnaires usually take less than 5 min each to complete but require the subject to be literate and have adequate vision. Patients who are not literate or who have poor vision can have the questionnaires administered verbally and this was necessary in a small proportion of the patients and controls.

All patients completed the questionnaires at their first visit and those for whom an underlying cause was identified following the results of investigations were subsequently excluded.

Statistical analyses of the differences between patients and controls were by chi-square tests, Mann–Whitney *U*-tests and Spearman-rank correlation tests as appropriate.

RESULTS

Chronic urticaria

The mean scores for the BDI and STAI are shown in Table 2. The mean BDI score was greater for urticaria patients than their controls but the difference in scores was not statistically significant. STAI scores were similar for urticaria patients and controls.

TABLE 1. Patient details

	Chronic urticaria		Generalized pruritus	
	Patients	Controls	Patients	Controls
n	34	68	34	68
Sex (m:f)	19:15	38:30	13:21	26:42
Mean age (range) years	45 (19–74)	45 (16–77)	58 (19–85)	57 (16–87)
Duration (range) years	3 (0·25–20)	—	2·4 (0·5–24)	—

TABLE 2. Mean Beck depression inventory and Speilberger state–trait anxiety scores for patients with chronic urticaria, generalized pruritus and controls (all differences not significant)

	Chronic urticaria		Generalized pruritus	
	Patients Mean(+ / − SD)	Controls Mean(+ / − SD)	Patients Mean(+ / − SD)	Controls Mean(+ / − SD)
BDI	8·3 (6·3)	6·3 (4·8)	9·4 (8·0)	8·0 (6·3)
State anxiety	36·4 (10·4)	36·0 (9·6)	36·4 (13·5)	35·5 (9·3)
Trait anxiety	38·2 (10·0)	38·0 (10·5)	40·2 (12·6)	36·5 (10·7)

BDI scores > 14 were observed in 14·7% of the chronic urticaria patients compared with 4·4% of controls. Scores > 21 were seen in 5·9% of urticaria patients compared with 1·5% of controls (Fig. 1). These differences were not statistically significant. None of the urticaria patients and one (1·5%) of the controls (whose BDI score was > 21) had reported their depressive symptoms to their family practitioner.

Similar proportions of chronic urticaria patients and controls had state and trait anxiety scores above the upper 90% probability limit for the general population (Fig. 2).

There was a highly significant correlation between BDI and trait anxiety scores for both urticaria patients and controls using the Spearman-rank correlation test ($r = 0{\cdot}63$, $P = 0{\cdot}0005$ and $r = 0{\cdot}43$, $P = 0{\cdot}0008$, respectively).

Idiopathic generalized pruritus

The mean scores for the BDI and STAI are shown in Table 2. The mean BDI and trait anxiety scores were greater for pruritus patients than controls but the difference in scores was not statistically significant. State anxiety scores were similar for pruritus patients and controls.

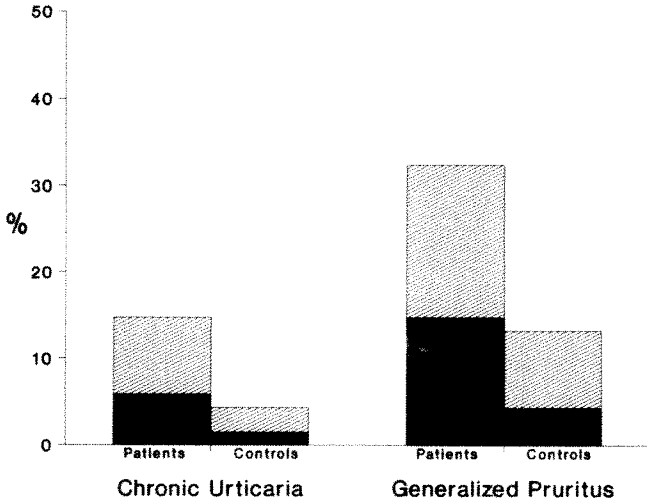

FIGURE 1. Beck depression inventory scores > 14 for patients with chronic urticaria and generalized pruritus and their controls. ▨, score 14–20; ■, score ≥ 21.

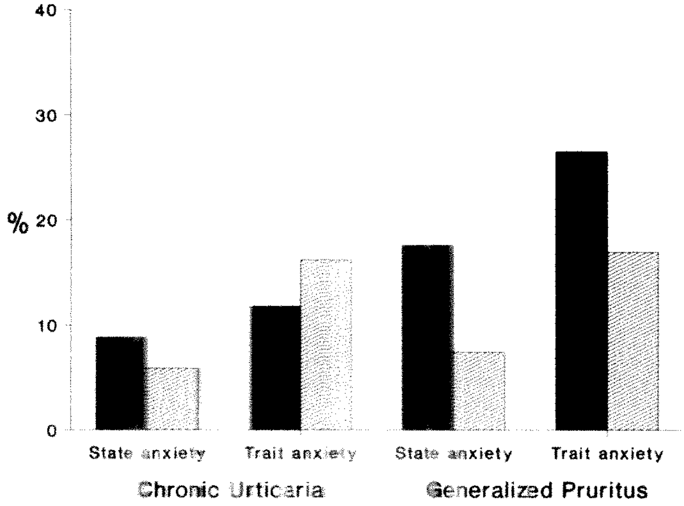

FIGURE 2. State and trait anxiety scores above the upper 90th probability limit for the general population for patients with chronic urticaria and generalized pruritus and their controls (all differences not significant). ■, patients; ▨, controls.

BDI scores >14 were observed in 32·4% of pruritus patients compared with 13·2% of controls (Fig. 1). This difference was statistically significant ($P<0·05$). Proportionately more pruritus patients had scores greater than 21 (14·7%) than controls (4·4%) but this difference was not statistically significant. Four (11·8%) of the pruritus patients and one (1·5%) of the controls had reported their depressive symptoms to their family practitioner or were under the care of a psychiatrist for depression and all had scores >21. None of the other depressed patients had reported their depressive symptoms to their family practitioner.

The proportions of pruritus patients with state and trait anxiety scores above the upper 90% probability limit for the general population were slightly greater than the controls (Fig. 2) but these differences were not statistically significant.

There was a highly significant correlation between BDI and trait anxiety scores for both pruritus patients and controls using the Spearman-rank correlation test ($r=0·53$, $P=0·003$ and $r=0·52$, $P=0·0001$). High trait anxiety scores were often associated with depressive symptomatology. The combination occurred in seven (20·6%) pruritus patients, four (11·8%) of whom had high depression scores (>21).

DISCUSSION

Many authors have considered psychological stress and emotional disturbances to be frequent in patients with chronic urticaria.[4] In a study of patients with a variety of skin diseases, Preston[1] reported 'obvious' or 'masked' depression in 94% of 17 patients with chronic urticaria. He also reported depression in over 50% of patients with each of the most common skin disorders. However, the criteria used to make these diagnoses were not clearly stated. Only a few studies have attempted to assess psychological problems in patients with chronic urticaria more objectively. Twenty patients with chronic urticaria were investigated using psychological questionnaires and an increase in symptom scores for anxiety and depression was found when compared with patients with fungal infections.[2] A similar increase in symptoms of anxiety and depression was also found in patients with psoriasis. The patients in this study were selected in that they were dermatology in-patients. Lyketsos *et al.*[3] investigated 28 patients with idiopathic urticaria using the present state examination schedule which is a structured clinical interview. They found depression in 29% and anxiety states in 68% of urticaria patients. Patients with psoriasis or alopecia had less anxiety but rather more depression. The patients were again selected in that they were dermatology in-patients. In the present study of unselected dermatology out-patients, we have found a lower incidence of depression and anxiety. Although depression was more common in urticaria patients than their controls, the difference was not statistically significant. The incidence of significant state and trait anxiety was similar to general dermatology out-patients and of the order which might be expected in the general population.

Patients with idiopathic pruritus have also been associated with psychological abnormalities. Idiopathic pruritus, particularly when localized to the anogenital area, has been associated with anxiety and depression.[4,5] However, there have been few controlled observations in patients with localized or generalized pruritus. We have found that patients with idiopathic generalized pruritus have a significantly increased incidence of depression compared with unselected general dermatology out-patients. The patients with depression frequently had significant anxiety symptoms. Although pruritus patients had a higher incidence of high state and trait anxiety scores, the difference was not statistically significant.

The levels of anxiety and depression indicated by these questionnaires should ideally have been confirmed by independent assessment by trained psychiatrists, with a clinical diagnosis given where appropriate. However, both questionnaires have been found to correlate well with the results of more formal psychiatric assessments.[9,10] The purpose of this study was to compare selected patient groups in terms of levels of anxiety and depression rather than formally diagnose psychiatric illness. The controls in this study were not matched for other factors such as socio-economic or marital status and this may have been a source of error in our results.

It is not possible to determine from the results of this study whether pruritus is a symptom of depression or whether the observed high incidence of depression merely represents a reaction to a persistent and distressing symptom. Whilst the patients in this study with pruritus and urticaria cannot be compared directly with each other because of differences in age and sex distribution, both groups showed an excess of depression when compared with their controls (albeit not statistically significant in the urticaria patients). It is possible that both disorders (and perhaps other dermatological conditions) have a propensity to produce depression. The reasons for the increased incidence of depression in both pruritus patients and their controls when compared with the urticaria group is unclear but may be due in part to differences in sex and age which are known to influence the incidence of depressive disorders.[11]

It is noteworthy that the majority of patients with urticaria or pruritus did not have significant symptoms of anxiety or depression. Although no firm conclusions can be drawn from this observation, it does argue against a major role for these forms of psychopathology in the pathogenesis of chronic urticaria and idiopathic generalized pruritus. Further work is required to determine the role of psychological factors in the pathogenesis of these conditions, and studies looking at the effects of treating those patients with significant levels of anxiety and depression may provide useful information.

The incidence of significant levels of depression and anxiety in dermatology out-patients in general, and particularly those with idiopathic generalized pruritus, is not inconsiderable. Patients frequently do not report their symptoms of anxiety and depression to their general practitioners. There is a need for an adequate psychological assessment of these patients by clinicians with a view to referring where appropriate for further treatment. Identification of significant depression or anxiety will provide an opportunity to reduce morbidity in these patients. The use of self-assessment questionnaires is a useful and simple way of screening for significant psychological problems, particularly depression.

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Clear ultraviolet blocking lenses for use by PUVA patients

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SUMMARY

It is well recognized that patients receiving photochemotherapy (PUVA) need to wear UV-blocking sunglasses on the day of ingestion of 8-methoxypsoralen. For many patients the wearing of tinted sun-glasses causes difficulties because they interfere with colour perception, reduce definition in conditions of low background light and often because they are considered 'cosmetically unacceptable'. In this study the UV-blocking properties of a number of lenses with little or no tint were assessed. The following lenses or lens coatings were found to be suitable for use by PUVA patients: Orcolite UV 400, Orma UVX, Rodenstock Lambda 400, Sola UV Gard 400 and Polaroid polarizing lenses.

According to animal studies,[1,2] one potential adverse effect of photochemotherapy (PUVA) is the premature formation of cataracts. As a result, close fitting goggles are essential during exposure within the PUVA cabinet. The 'at risk' period, however, persists for approximately 24 h following ingestion of the psoralen and during this time patients are advised to wear UV-blocking sunglasses when outdoors. A recent survey of 58 pairs of sun-glasses used by 53 PUVA patients,[3] however, showed that whilst all patients were wearing tinted spectacles, a number of these were unsatisfactory, emphasizing the need for dermatologists to recommend specific spectacles, not just 'tinted sun-glasses'.

The wearing of sun-glasses, however, has a number of potential problems. Firstly, most are tinted and are likely to interfere with colour perception which may pose difficulties for those who need to distinguish colours in the course of their work. A further problem, which again may interfere with a patient's work is that tinted spectacles reduce definition in conditions of low ambient light. Finally, a number of patients find wearing tinted sun-glasses (especially in the winter) cosmetically unacceptable, removing the spectacles as soon as they leave the hospital premises. We have investigated the suitability for PUVA patients of glasses incorporating UV

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blocking lenses with little or no coloured tint, these being cosmetically more acceptable and not interfering to any significant degree with the transmission of visible light.

In this paper we report on the transmission characteristics of uncoloured ophthalmic lenses or lens coatings which, according to the suppliers, have ultraviolet blocking properties. The results obtained are compared with criteria considered acceptable for PUVA patients which have been previously published.[3]

METHODS

A number of 'UV-blocking' lenses which contain little or no colour tint were obtained from ophthalmic opticians and lens suppliers. Some of these were lenses impregnated in manufacture with a UV-absorbing material, others being coatings applied to existing lenses. The ultraviolet and visible radiation transmission characteristics of these lenses were then assessed between 250 and 800 nm using methods described elsewhere.[4,5] A deuterium lamp with an ultraviolet spectroradiometer and a tungsten filament lamp with a visible radiation spectroradiometer were used to cover the spectral range. The criteria used to determine suitability of the various lenses for PUVA patients were those defined previously[3] (Table 1), additional criteria being that the lenses or coatings should be readily available and cosmetically acceptable.

RESULTS

The transmission results from 12 of the lenses investigated are shown in Figures 1 and 2. The Orcolite UV 400 (Norville Optical Ltd), the Orma UVX (Essilor Ltd) and the Perfalit Lambda 400 coated lenses (Rodenstock Ltd) and the Sola UV Gard impregnated lenses (Pilkington Ophthalmic Opticians) all satisfied the criteria of acceptability as did Polaroid polarizing lenses. In contrast, however, the RXH12, UV and Rose lenses (Hoya Ltd), Companion tint C84, R85 and G88 lenses (Norville Ltd), and Cam-lite coated lenses (Cambridge Optical Ltd) did not meet the criteria. Transmission measurements were also carried out on a plastic (CR39) ophthalmic lens before and after coating with Orcolite UV 400 (Fig. 3). This demonstrated the effectiveness of the coating which rendered a previously unsuitable lens acceptable according to our criteria.

DISCUSSION

Previous studies have shown that free methoxypsoralen diffuses out of the rat lens within 24 h,[6] provided the animal is kept in a darkened environment. Exposure to UV-radiation during this

TABLE 1. Transmission limits of lenses for use by PUVA patients

Wavelength	Transmission limit (%)
390	10
380	5
370	2
360 and below	1

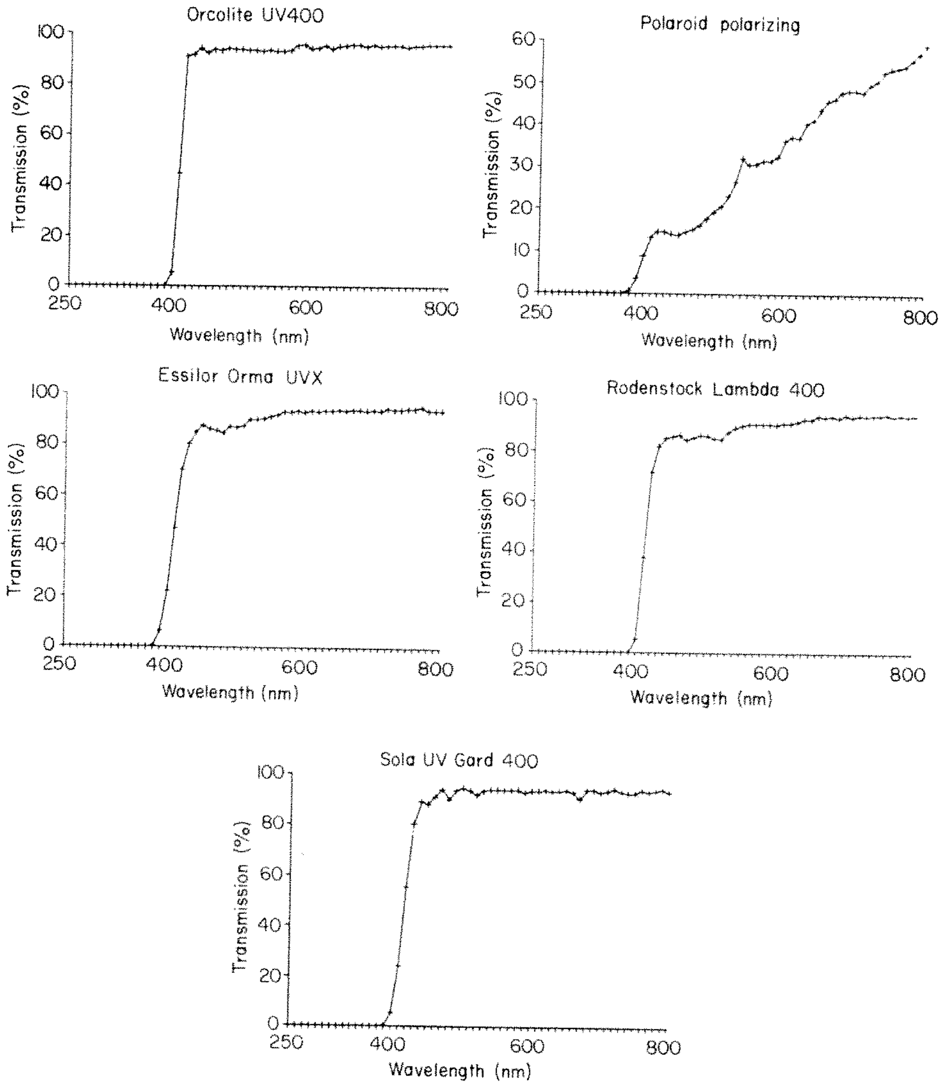

FIGURE 1. Transmission characteristics of lenses investigated in the present study which satisfied the criteria of acceptability.

period, however, causes the formation of photo-addition products which are fixed within the lens. In man, free methoxypsoralen has been demonstrated in the human lens up to 12 h following ingestion of oral methoxypsoralen[6] and evidence that these psoralens may induce cataracts comes from the finding of psoralen photo-products in cataract material from patients who had received PUVA therapy, but who had not worn protective eyewear.[7,8] It is, therefore, advisable for PUVA patients to wear sunglasses or spectacles which effectively block ultraviolet radiation for a period of 24 h after oral ingestion of psoralen.

Perhaps the most important factor which determines whether such spectacles are effective is patient compliance, and it is largely for this reason (as well as the fact that UV reaching the eye

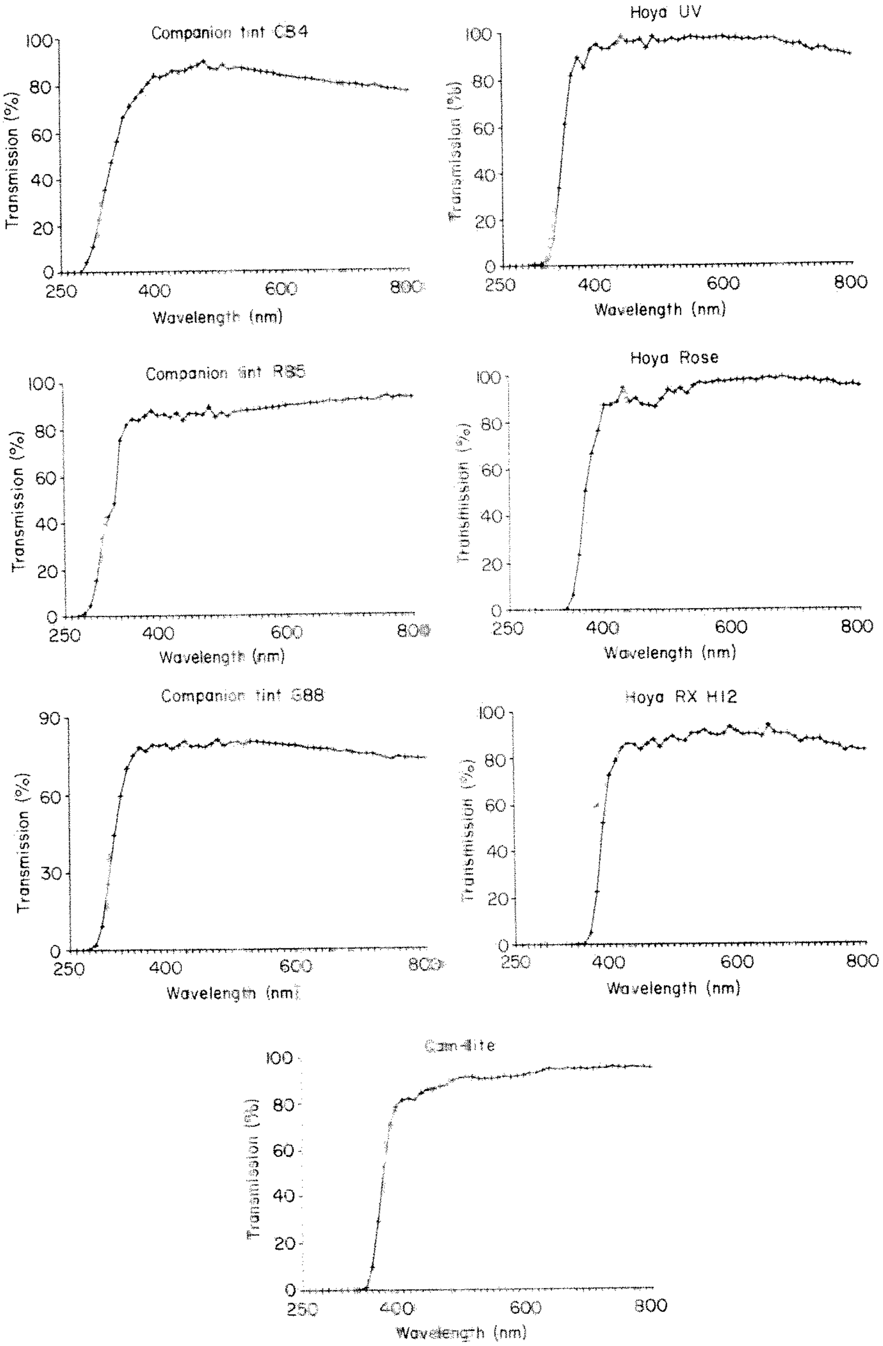

FIGURE 2. Transmission characteristics of other lenses investigated in the present study.

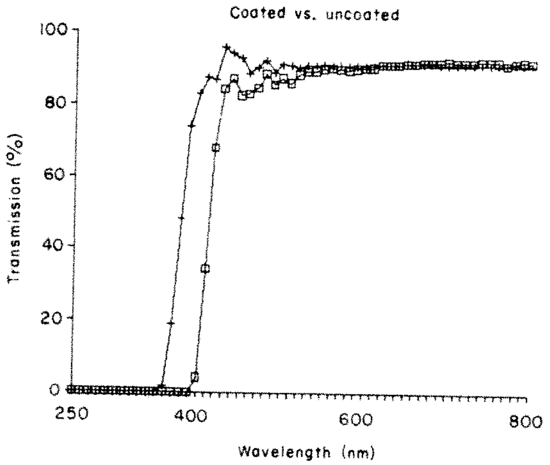

FIGURE 3. Comparison of spectacle lenses, one coated with Orcolite UV 400 (—□—) and the other uncoated (+).

TABLE 2. Lenses recommended for use by PUVA patients

Lenses treated with Orcolite UV 400
Essilor Orma UVX lenses
Sola UV Gard 400 lenses
Rodenstock Perfalit Lambda 400 lenses
Polaroid polarizing lenses

TABLE 3. Ultraviolet radiation transmittance levels according to various standards for lenses which transmit 80% of visible radiation

Standard	UV range (nm)	Maximum transmittance (%)	Notes
BS 2724	315–400	Tv	Shade no. 1.1
	280–315	0·1 Tv	
EN 170	365–400	Tv	
	313–365	50	Scale no. 3–1.2
	313–365	0·3	Scale no. 2–1.2
	210–313	0·0003	
ANSI Z80.3	315–400	Tv	
	290–315	30	

Tv, luminous transmittance (which relates closely to the transmittance of visible radiation).

round the side of sun-glasses is relatively small in relation to that passing through the spectacle lens) that wrap around eyewear has not been widely recommended for use by PUVA patients. Even normal tinted sun-glasses, however, render PUVA patients 'out of the ordinary', especially in the winter, thus contributing to poor compliance.

The clear lenses and lens coatings which we have found to meet our optical criteria and which are also cosmetically acceptable in terms of tint are shown in Table 2. Orcolite UV 400, Orma UV-X and Perfalit Lambda 400 are UV-absorbing coatings which are applied to plastic (CR39) lenses. All of these coatings can be applied to prescription lenses or sun-glasses and are widely available from opticians. However, the lenses must be in near-perfect condition. The Perfalit Lambda 400 incorporates a pale brown tint. Unfortunately, the coatings cannot be applied to glass lenses. One clear 'impregnated' lens was found to be suitable, the Sola UV Gard 400. This is manufactured from a hard resin (CR39) impregnated with a UV blocker during manufacture. Also listed in Table 2 are Polaroid polarizing lenses (not all 'Polaroid' lenses are polarizing) which contain an efficient UV blocker cast into the base material. Although these lenses are not totally clear, they are optically acceptable for PUVA patients and are considered cosmetically acceptable by many. It should be emphasized that untreated CR39 is unsuitable for use by PUVA patients, the spectral transmission results obtained in this study being similar to previously published spectra.[9]

The above recommendations are based on the criteria outlined in Table 1. There are also product standards for sunglasses, British Standard 2724,[10] European Standard 170,[11] and American National Standard Z80.3.[12] Unfortunately, we consider these specifications to be inappropriate for the special requirements of PUVA patients. The product standards are fairly complex but for lenses with high luminous transmittance, essentially, they require that the transmission of UV-radiation should not exceed that of visible light. This is illustrated by the example (Table 3) of a lens which transmits 80% of visible light. Such a lens could also transmit 80% of UV-radiation down to a wavelength of 365 nm in the case of EN 170 and even as far as 315 nm for compliance with BS2724 and ANSI Z80.3.

Finally, we would consider it advisable that patients with existing plastic spectacles should have them coated through hospital optical departments who are aware of these patients' special needs and who can ensure that the proper coatings are applied. If patients do go to 'high street' opticians, they must insist that their spectacles are sent for the specific coatings recommended. Even then, the result is not always predictable: one reputable 'high street' optician recommended one of the authors to have the Orcolite UV 400 coating applied to his spectacles; on return, they did not meet our transmission criteria, not unexpectedly, as the lenses were glass and, therefore, could not have been coated with what had been asked for.

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Factor XIIIa in nodular malignant melanoma and Spitz naevi

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SUMMARY

The distribution of factor XIIIa-positive dermal dendritic cells was studied in a series of nodular malignant melanomas and compared with that seen in Spitz naevi. Two patterns of distribution were recognizable: (a) diffusely spread through the tumour and (b) located mainly at the periphery of the tumour. These did not correlate with the diagnosis of melanoma or Spitz naevus and the distribution appeared to be a function of growth pattern of the tumour. The diffuse pattern was the most common regardless of diagnosis and the distribution of factor XIIIa-positive cells is the same in malignant melanoma and Spitz naevi.

The clinical differentiation of Spitz naevi from malignant melanoma is of great significance for the patient but is often problematic. The distinction at the light microscopic level using routine stains can be difficult[1–5] and although special techniques such as the silver-staining nucleolar-organizer-region (AgNOR) method show a clear distinction between groups of the two lesions, they are unhelpful in individual cases.[6] The problem is the lack of a marker for the difference between a benign and a malignant melanocyte. We have recently studied the distribution of a group of dendritic dermal cells that express an epitope that reacts with a polyclonal antibody to the blood clotting substance factor XIIIa (FXIIIa). These cells have been shown to be present in the dermis of normal skin and they are variously thought to be related to fibroblasts[7] or histiocytes.[8,9] Cerio *et al.*[7] found that the cells did not label with Mac 387, a macrophage marker, supporting the views of other groups who favour a fibroblastic origin.[10] However, other studies using double-staining techniques seem to support a monocyte–macrophage lineage.[3]

In our preliminary studies on these cells we looked at a series of inflammatory skin specimens and a number of tumours including several melanocytic lesions. We found that the distribution of FXIIIa positive cells was different in two Spitz naevi (diffuse) and two malignant melanomas (peripheral) and thought it would be of value to compare a much larger series.

METHODS

Cases of nodular malignant melanoma and of Spitz naevi were retrieved from the files of the

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Pathology Department of the Royal Hallamshire Hospital. Sections stained with haematoxylin and eosin were reviewed and only those cases considered to be 'classical' examples were accepted. Ten sections of each were used for FXIIIa staining. The sections from paraffin blocks were cut at 5 μm, dewaxed and the endogenous peroxidase activity blocked using 0·5% hydrogen peroxide in methanol. Trypsin digestion for each block was empirically determined for optimum time,[11] using a 0·1% trypsin solution (Sigma) in calcium chloride at 37°C. The times varied between 2 and 8 min, and were used prior to the primary antibody incubation for demonstration of subsequently antigenic sites.

The rabbit antibody to factor XIIIa (Behring) was tested on control material in order to determine its optimum dilution: 1:500, and incubation conditions: 0·5 h at room temperature. Each subsequent stage had 0·5 h incubation time, with a goat anti-rabbit immunoglobulin bridge (Atlantic Antibodies) followed by a rabbit peroxidase anti-peroxidase conjugate,[12] both at a 1:400 dilution, with 0·5 h TBS washes between all stages. The resulting bound peroxidase was detected using the diaminobenzidene–hydrogen peroxidase reaction.[13] Sections were counterstained with haematoxylin.

Negative controls were included and consisted of normal rabbit serum substituted for the primary rabbit antibody, omission of the primary antibody and omission of the primary antibody and link stages. Positive controls for the primary antibody were also included.

RESULTS

The distribution of dermal dendrocytes within the lesions seemed to fall into two categories: those with a diffuse distribution (Fig. 1) and those where staining was concentrated at the periphery of the lesion (Fig. 2). No formal attempt was made to quantitate FXIIIa positive cells but no gross differences in numbers were apparent.

Two observers reviewed the immunoperoxidase-stained section without knowing the histological diagnosis and placed them into these two categories (predominantly diffuse or

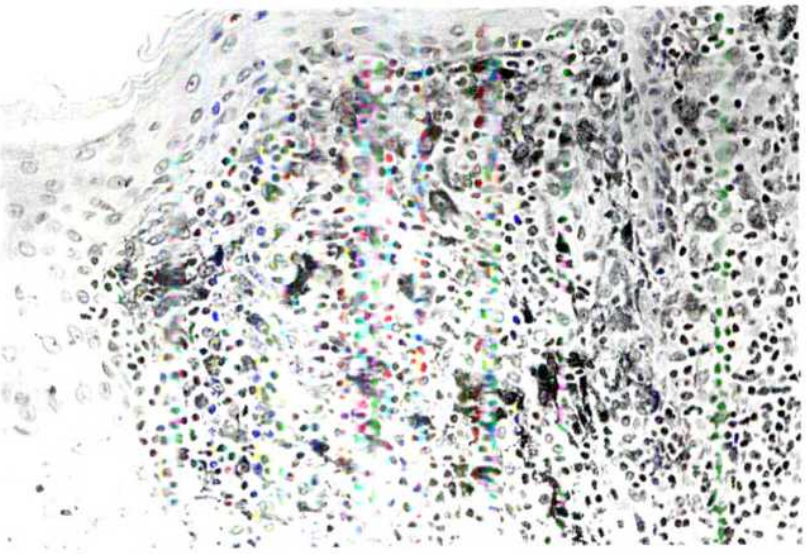

FIGURE 1. A diffuse pattern of distribution of FXIIIa positive cells in a malignant melanoma (× 175).

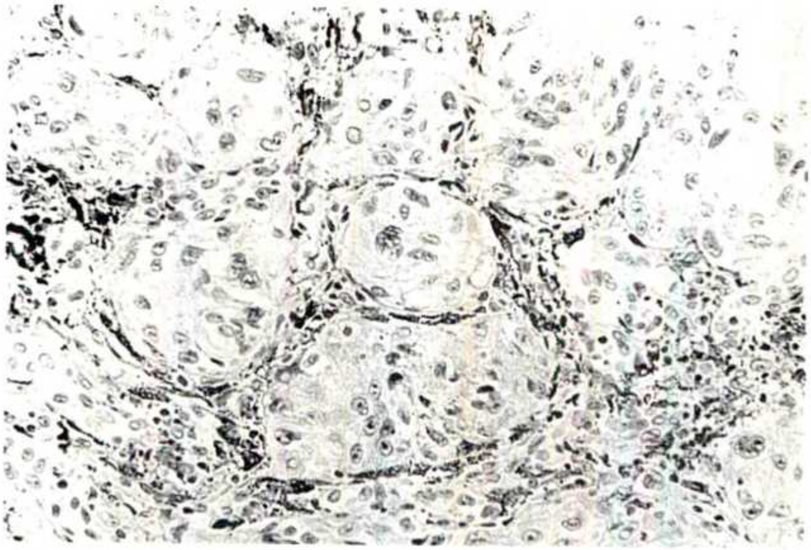

FIGURE 2. A peripheral pattern of distribution of FXIIIa positive cells in a malignant melanoma (× 175).

TABLE 1. Classification of the distribution of Factor XIIIa positive cells in melanoma and Spitz naevus into predominantly diffuse or peripheral as determined by two observers

	Observers	
Diagnosis	1	2
Melanoma		
Diffuse	10	7
Peripheral	3	3
Spitz naevus		
Diffuse	5	6
Peripheral	5	3

peripheral) (Table 1). Observer 1 found two unclassifiable and observer 2 found six unclassifiable.

DISCUSSION

Using an antibody specific for factor XIIIa we demonstrated that the distribution of the normal population of dendritic dermal cells was affected by the presence of a Spitz naevus or melanoma. However, the resulting patterns of distribution of these cells were not useful in differentiating between the two lesions. It has been suggested that the factor XIIIa positive dermal dendrocyte is derived from cells of the monocyte/macrophage series. If this were so they might be expected to have a function as antigen-presenting cells. A functional significance would be implied by a constant reaction to an antigenic stimulus such as a neoplastic proliferation. The absence of such an effect adds to the evidence against a monocyte/macrophage origin for these cells.

The distribution of FXIIIa positive cells in these pigmented lesions falls into two patterns similar to that seen in dermatofibromas and histiocytomas.[7] Our interpretation of these patterns in pigmented lesions is that both melanomas and Spitz naevi grow as diffuse or expansile lesions. In the former the melanocytes become mixed with the FXIIIa positive cells and in the latter type of growth pattern they compress the dermal dendrocytes into a rim at the edge of the lesion.

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Topical treatment of necrotic foot ulcers in diabetic patients: a comparative trial of DuoDerm and MeZinc

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SUMMARY

An open randomized controlled study was carried out of 44 diabetic patients with necrotic foot ulcers treated with adhesive zinc oxide tape (MeZinc) or with an adhesive occlusive hydrocolloid dressing (DuoDerm). Fourteen of the 21 patients treated with MeZinc had their necrotic ulcers improved by at least 50% compared to six out of 21 with the hydrocolloid dressing ($P < 0{\cdot}025$). Fifteen patients showed an increase in the area of necrosis during the course of the 5-week study and of these, 10 had been treated with the hydrocolloid dressing.

Ulceration of the foot is a common complication in patients with diabetes mellitus[1–3] and they often take a long time to heal.[4] Some of these lesions will progress to gangrene and severe infections[5,6] that may require minor or major amputations.[7–9] There are few controlled studies on the topical therapy of foot ulcers in diabetics, especially on those that are necrotic. In recent years occlusive hydrocolloid dressings have been increasingly used in the management of ulcers to improve wound healing,[10] and experimental studies on full thickness wounds indicate that they are effective in necrotic lesions.[11,12] Topical zinc oxide is used in the treatment of superficial ulcers[13,14] and its beneficial effects have also been reported in the treatment of necrotic burns.[15]

This study was carried out to investigate whether topical occlusive treatment of necrotic ulcers on the feet of diabetics could achieve early separation of the necrotic eschar without damage to the viable tissue. It was also carried out to compare the adhesive zinc oxide tape (MeZinc) with an occlusive hydrocolloid dressing (DuoDerm) as to their capacity in dissolving the necrotic tissue in these ulcers.

METHODS

Patients

Forty-four patients with diabetes mellitus were included in this study because of necrotic foot ulcers. All the patients were investigated and treated as out-patients with a combined foot-care

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team. There were 26 men and 18 women and the mean age was 63 years (range 23–86 years). The mean duration of their diabetes was 20 years (range 2–54 years). Thirty-five patients were treated with insulin, eight with oral hypoglycaemics and one with diet alone. Onset of diabetes occurred in 17 of the patients before the age of 30. The mean duration of the foot ulcers before being included in the study was 9 weeks (range 1–105 weeks).

Study design

This was an open controlled trial for 5 weeks with blinded evaluation. The patients were randomly allocated to treatment either with MeZinc (Mölnlycke Health Care, Sweden) or DuoDerm (ConVatec, U.S.A.). Duoderm is also known as Granuflex or Varhesive in certain countries.

Criteria for inclusion and exclusion

The criteria for inclusion were previous diabetes mellitus and a superficial full thickness skin ulcer below the ankle[16] and with a systolic toe pressure above 45 mmHg or an absence of cutaneous erythema.[17,18] The ulcers were between 1–25 cm^2 in area with more than 50% of the area covered with dry or wet necrotic tissue. A wet necrotic ulcer was considered to be present when more than half of the area was moist. Only one ulcer was studied in each patient and if there were several, the largest one was chosen.

Patch tests were performed before the patients were included in the study and those with positive tests were excluded, as were those showing clinical signs of cellulitis. Ulcers, where the application of these dressing was inappropriate, were also excluded.

Treatment

Patients were seen weekly by a combined foot-care team that consisted of a diabetologist, an orthopaedic surgeon, an orthotist, a podiatrist and a nurse. All patients were offered the same basic treatment during the study. The foot wear was corrected when necessary and external pressure on the ulcer relieved.[19] The ulcers were cleaned with sterile saline and dressed according to the manufacturers' recommendations by the regular health care nurses. Dressings were changed daily during the first week and every 3 days afterwards. The patient was asked each time about possible side-effects and the wound and surrounding area inspected and assessed. During the study there was no surgical debridement. The treatment was stopped in those cases that were hospitalized or where there was a lack of compliance, and also in cases where there was surgical debridement or a marked increase in the size of the ulcer or the area of necrosis and where there were local reactions caused by the dressings.

Wound evaluation

Colour photographs were taken at each weekly visit and the ulcer and area of necrosis measured. At the end of the study the photographs were evaluated by two separate observers. A successful result was where the initial necrotic area was either totally dissolved or reduced by at least 50% of the initial area.[20] Failure of treatment was where there was an increase in the area of necrosis or ulcer of 50% or more.

Laboratory investigations

The systolic ankle and toe pressure were measured using the strain-gauge technique as previously described.[18] The systolic ankle pressure was measured in the tibial or dorsalis pedis arteries. Control of diabetes was assessed by HbA$_{1c}$ levels of fasting venous blood glucose levels both at the beginning and the end of the trial. Plasma zinc was also measured under fasting conditions.[21]

Statistics

Differences were calculated using the χ^2-test, Mann–Whitney (two-tailed) and Wilcoxon rank-sum tests.

RESULTS

Fourty-four patients were included in the study and the details are shown in Table 1. Both groups of patients were comparable though there were more men in the group of patients treated with DuoDerm. Thirty-one of the 44 necrotic ulcers were considered to be dry (Table 2) and the remaining 13 wet. The location and size of the ulcers was much the same in the two groups.

TABLE 1. Patient characteristics at enrolment

	MeZinc ($n = 22$)	DuoDerm ($n = 22$)
Age (years)	63 (13)	62 (18)
Sex (men/women)	10/12	16/8
Duration of diabetes (years)	22 (15)	19 (12)
Diet	1	—
Oral hypoglycaemic agents	4	4
Insulin	17	18
Systolic toe pressure (mmHg)	66 (33)	68 (32)
Systolic ankle pressure (mmHg)	104 (41)	114 (52)
fB-glukos (mmol/l)	9·1 (4·8)	9·0 (4·0)
HbA$_1$c (%)	8·4 (1·4)	8·0 (2·1)
Zinc (μg/ml)	0·74 (0·05)	0·76 (0·05)

Values indicate means (± SD).

TABLE 2. Ulcer characteristics at enrolment

	MeZinc ($n = 22$)	DuoDerm ($n = 22$)
Initial ulcer area (cm^2)	2·2 (1–10·5)	2·2 (0·9–20·4)
Initial necrotic area (cm^2)	1·5 (0·5–10·5)	1·6 (0·9–19·2)
Dry necrotic ulcer	15	16
Wet necrotic ulcer	7	6
Localization		
Dig I	5	4
Dig II-V	3	3
Plantar surface	1	—
Dorsal area	1	2
Malleolus	7	8
Heel	5	5

Areas are given as median values and range.

Evaluation of necrotic tissue

Either total disappearance or reduction of > 50% of the initial necrotic area was seen in 20 of the patients, 14 of those treated with MeZinc and six with DuoDerm (Table 3). In nine of the patients there was an increase in the area of necrosis of more than 50%. This occurred in four patients treated with MeZinc and five with DuoDerm. Treatment had to be discontinued in eight of these patients because of an increase of the necrotic area by more than 100%. The changes in the areas of necrosis in those patients completing 5 weeks' trial are shown in Figure 1. The patients treated with MeZinc decreased their relative necrotic area during the course of the study but this was not seen in those treated with DuoDerm.

Laboratory studies

There was no significant difference in the results of investigations carried out in the two groups.

TABLE 3. Changes in necrotic ulcer area

	MeZinc ($n=22$)	DuoDerm ($n=22$)
No remaining necrosis	9	5
Decrease > 50%	5	1
Decrease 25–50%	1	2
No change (± 25%)	1	3
Increase 25–50%	1	5
Increase > 50%	4	5
Excluded	1	1

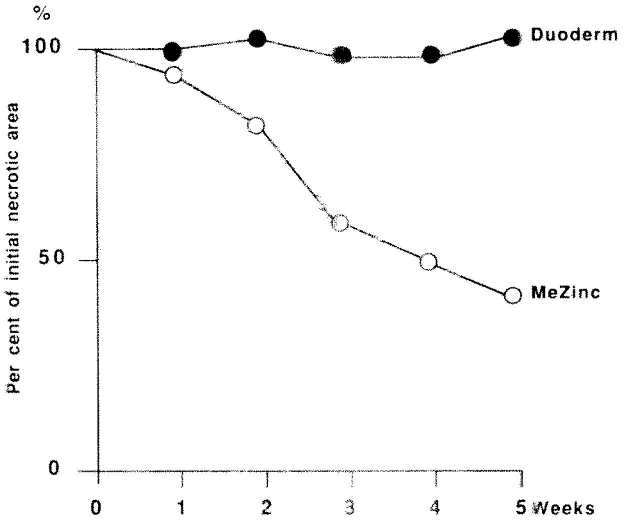

FIGURE 1. Mean changes in area of necrosis in patients treated for 5 weeks with DuoDerm ($n=17$) or MeZinc ($n=18$).

Adverse reactions

Maceration of the skin edges was seen in both groups. Four patients treated with MeZinc had to discontinue treatment because of an increase in the area of necrosis and this was associated with pain and oedema. Five patients treated with DuoDerm had a similar increase in the area of necrosis. One patient after 5 weeks of treatment with DuoDerm showed signs of cellulitis and this was due to *Staphylococcus aureus*. This patient was treated with systemic antibiotics and the DuoDerm dressings discontinued. Compliance by the patients in this study was excellent.

DISCUSSION

In this controlled study two types of dressing were compared in the treatment of necrotic foot ulcers in patients with diabetes. Only 20 of the 44 patients showed an improvement of their necrotic ulcers. Thus, treatment with occlusive dressings are not advocated for this type of foot lesion in diabetes. A common adverse effect seen in both groups was maceration of the skin edges and a possible explanation for this is the polyneuropathy which is almost invariable in diabetic patients with foot ulcers.[22,23]

Occlusive hydrocolloid dressings such as DuoDerm improve wound healing in superficial ulcers[10] and their use has been advocated for necrotic lesions.[11,12] However, this study does not support the use of adhesive occlusive hydrocolloid dressings for necrotic foot ulcers as only six of our 21 patients treated with the dressing had a decrease in size of the area of necrosis. Furthermore in almost half the patients treated with DuoDerm there was an increase in the area of necrosis. These patients had a tendency towards higher HbA_1c levels and a lower systolic toe pressure and a greater initial ulcer size and area of necrosis compared with those who improved during the course of the study. The number of patients, however, is too small to allow any definite conclusions, though these findings are in agreement with previous reports concerning wound healing in diabetic patients.[4,13,18,20,24] The advantages and disadvantages of hydrocolloid dressings for treatment of foot ulcers in diabetes have been previously discussed.[25]

Topical treatment with zinc oxide has been shown to be beneficial where wound healing was delayed in cases of zinc deficiency, and in full thickness burns and superficial leg ulcers.[13,14] It has also been of help in necrotic ulcers not specifically reported in diabetic patients.[15,26] In this study 14 of the 21 patients treated with MeZinc had a reduction or disappearance of the areas of necrosis in the 5-week period. However, four of the patients had an increase in the size of the necrotic area with an increase of local pain and oedema. The mechanism of action of zinc in producing wound healing is unknown though various possibilities have been suggested.[13]

In conclusion, from this study we cannot recommend the use of adhesive hydrocolloid dressings such as DuoDerm for the treatment of necrotic ulcers on the feet of diabetics. Although an adhesive zinc oxide tape reduced the initial necrotic area in many of our diabetic patients, this treatment is not without risk and should be used for necrosis that is limited to the skin and does not involve deeper tissue.

ACKNOWLEDGMENTS

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Perianal streptococcal cellulitis with penile involvement

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SUMMARY

Perianal streptococcal cellulitis is described occurring in a 5-year-old boy. The condition also involved the penis and presented as a possible case of sexual abuse. The correct diagnosis was established by culturing β-haemolytic streptococci group A from the penile and perianal skin.

Perianal streptococcal cellulitis was first described by Amren *et al.*[1] from the U.S.A. and further cases have been reported from the same country.[2-4] The condition is caused by β-haemolytic streptococcus group A and is characterized by a well-demarcated bright erythema around the anus, without satellite lesions. The symptoms include painful defaecation with secondary constipation, rectal bleeding and a brown mucoid anal discharge. The condition has been mainly reported in prepubertal children and there is an unexplained male predominence. We describe a case of perianal streptococcal cellulitis which was complicated by penile involvement and mistaken for sexual abuse.

CASE REPORT

A 5-year-old boy was referred to hospital with a 4-week history of painful defaecation and micturition, passing blood-stained stools and with erythema of the perianal and preputial areas. Sexual abuse was suspected when the symptoms failed to resolve following appropriate treatment for threadworms, candidiasis, psoriasis and eczema. His social history revealed that his parents had divorced shortly after his birth and that he lived during the week days with his 22-year-old mother, her boyfriend and two younger brothers, and spent weekends with his father. He had been encouraged to retract his foreskin as he had a narrow prepuce, although this had not interfered with micturition. One of his brothers had been circumcized for phimosis.

Examination revealed a livid erythema encircling the anus 1–3 cm in distance from the anal margin with an overlying whitish exudate (Fig. 1), minor anal fissures and brown staining of the

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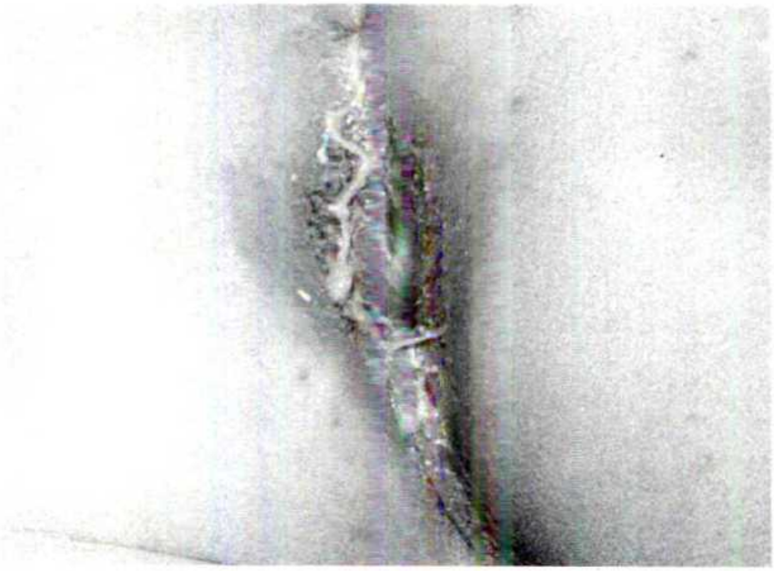

FIGURE 1. Well demarcated perianal erythema with overlying exudate.

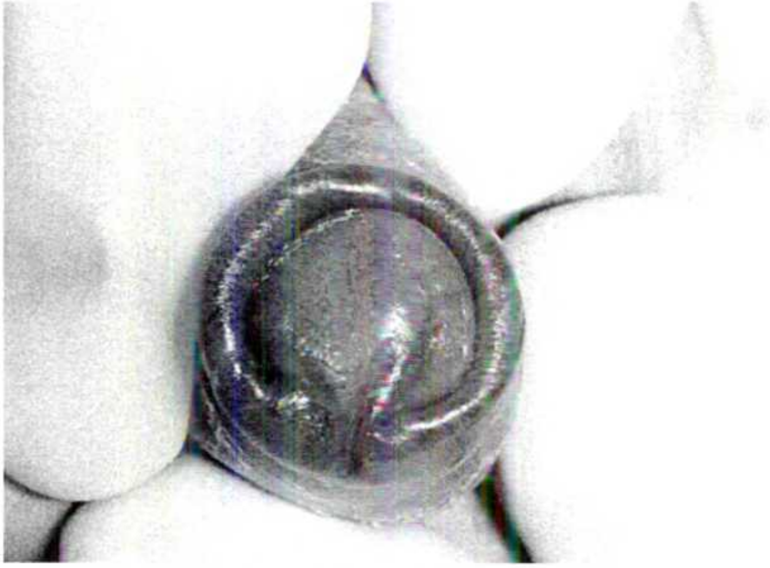

FIGURE 2. Erythema and swelling of prepuce and glans penis.

underpants. In addition, there was intense erythema of the glans penis and the prepuce (Fig. 2), and the latter was also oedematous. The rest of the skin was normal. His brothers were unaffected, but other family members declined a physical examination.

An initial swab from the perianal region was sterile on culture following inoculation on selective media. However, a swab from the tip of the penis yielded a heavy growth of β-

haemolytic streptococci group A when inoculated on 5% horse blood agar. *Candida albicans* and *Neisseriae gonorrhoeae* were not isolated from either site. A subsequent perianal swab inoculated on 5% horse blood agar also yielded a growth of β-haemolytic streptococci group A. Serotyping of streptococi revealed 'T' type 28 and 'R' type 28 from both the perianal and penile lesions. Swabs taken from the throat, anterior nares, abdomen and the fingers of the patient were sterile, as were swabs from the throat, penis and perianal areas of his brothers. Serological studies on the patient demonstrated anti-streptolysin 'O' titre 480 IU/ml (N < 240 IU/ml); anti-DNase B titre 1920 IU/ml (N < 240 IU/ml) and anti-hyaluronidase titre 128 IU/ml (N < 256 IU/ml).

Treatment with oral phenoxymethyl penicillin 500 mg q.i.d. resulted in clearance of the penile lesion after 14 days, but the perianal lesion persisted and streptococci were isolated from this site despite 3 weeks of antibiotic therapy. A further 2 weeks of penicillin therapy was required to eradicate streptococci and clear the perianal erythema. An investigation by social workers did not reveal any conclusive evidence of sexual abuse.

It is of interest that the patient's father, mother and her boyfriend all had a severe sore throat 2 weeks prior to the onset of perianal symptoms in the patient. Throat swabs were not taken but antibiotic therapy resulted in resolution of symptoms within 10 days. However, his mother was 4 months pregnant, did not receive antibiotic therapy and her symptoms took 3 weeks to resolve. The patient and his brothers did not complain of a sore throat.

DISCUSSION

It was fortunate that the above patient had penile involvement from which β-haemolytic streptococci were isolated, as the correct diagnosis would otherwise have been missed and a label of sexual abuse difficult to remove. Previous reports[1,3] have stressed the need for clinicians to request inoculation of perianal swabs on blood agar for the isolation of β-haemolytic streptococci as most microbiology departments routinely process such specimens on selective media designed to identify the common enteric pathogens. The failure to recognize perianal streptococcal cellulitis as a disease entity and the tacit acceptance of the results of culture on selective media probably accounts for the apparent rarity of this condition.

Early treatment with penicillin is essential as it is curative and avoids months of troublesome symptoms.[3] Antibiotic therapy needs to be continued for at least 2 weeks, until there is clinical clearance and repeat cultures show eradication of streptococci. It is unclear why the condition responds so slowly to oral or intramuscular penicillin therapy despite *in vitro* tests clearly demonstrating sensitivity of cultured streptococci to the antibiotic. It has been suggested that penicillinase produced by enterobacteria may serve to reduce the effective local concentration of penicillin. However, these organisms were not isolated on culture of a perianal swab from the above patient and previous reports have shown that treatment with erythromycin, which would not be affected by penicillinase, is no more effective than penicillin.[1] The mode of transmission of streptococci to the perianal area is also unclear, but a recent history of sore throat in family members has been noted previously.[4,5] Another potential source of group A streptococci is an asymptomatic anal carrier,[6,7] but it was not possible to assess the carrier state of the adults looking after the above patients as they declined an examination. The isolation of the same strain of streptococci from the penis and perianal area in the above patient, with apparent sparing of the skin between these sites, suggests a role for autoinoculation. It is of interest that penile involvement has not been previously described in boys with perianal steptococcal cellulitis although an associated streptococcal vulvovaginitis has been documented in prepubertal girls.[1,8] It is possible that traumatization of the foreskin during retraction in our patient may have predisposed the distal part of the penis to the development of this complication.

The differential diagnosis of perianal streptococcal cellulitis includes psoriasis, eczema, threadworms, candidiasis, inflammatory bowel disease and behavioural disorder.[2] In addition, the above case clearly shows that perianal streptococcal cellulitis may also masquerade as sexual abuse, and that confirmation of the correct diagnosis is by the simple expedient of sending a perianal swab for inoculation on blood agar.

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Erythema multiforme major in a 2-month-old child with human immunodeficiency virus (HIV) infection

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SUMMARY

A case is presented of severe erythema multiforme occurring in a 2-month-old boy from Zimbabwe which was probably triggered by phenobarbitone. He later developed the acquired immunodeficiency syndrome and serological tests suggested that it was a congenital HIV-1 infection.

Erythema multiforme (EM) both minor and major are acute self-limiting diseases of the skin and mucous membranes.[1] Although EM minor has been reported in children under the age of 1,[2,3] EM major and drug-induced toxic epidermal necrolysis (TEN) have rarely been reported in infants under the age of 3 months. We report a child with HIV infection who developed EM major that was likely to have been drug-induced.

CASE REPORT

A 2-month-old Negro boy, born in Zimbabwe, was admitted to hospital with a 3-day history of an erythematous rash associated with fever and bilateral conjunctivitis. He had been treated prior to the appearance of the rash with a syrup that contained phenobarbitone and pipenzolate bromide. The white cell count was 7900×10^9/l (25·5% non-segmented polymorphonuclear cells). The chest X-ray was normal. Cultures of blood, urine and cerebrospinal fluid were sterile and serological tests for *Mycoplasma pneumoniae*, measles, rubella and picornavirus were negative as well as immunofluorescence test and cultures for herpes simplex I and II and varicella zoster. Although there was no evidence clinically of infection, he was treated with cloxacillin and gentamicin. The following day the child developed iris-shaped papules with

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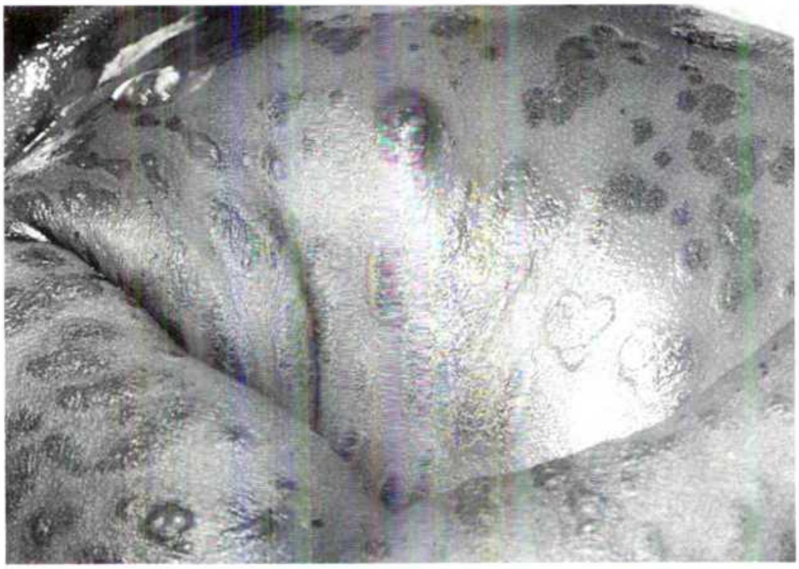

FIGURE 1. Bullae on arms and iris-shaped lesions on chest.

numerous bullous and erosive lesions (Fig 1) and involvement around the genitalia and mouth. Treatment with antibiotics was discontinued but there was no change as regards the skin lesions. A positive Nikolsky sign was noted. Histology of the roof of a blister showed necrosis of the entire epidermis. Treatment with a pulse of a single dose of 40 mg methyl-prednisolone was given over 1 h and during the infusion, he had a short episode of tachycardia and tachypnoea, but no other side-effects were observed. Within 24 h there was dramatic improvement and he was fully recovered within 5 days.

Over the next 3 months the child was well but was then admitted because of progressive weight loss associated with respiratory symptoms and signs. A severe bilateral interstitial pneumonitis was diagnosed. Investigation of the bronchiolar secretions for *Pneumocytis carinii* and *Mycoplasma pneumoniae* was negative. The child was found to be positive for HIV-1 by the ELISA test that was confirmed by immunoblot and gp 24 antigen was detected in the serum. The child developed thrombocytopenic purpura and died 1 week later. A post-mortem examination was refused by the parents who also declined to be tested for HIV infection. Positive serology for HIV-1 was demonstrated retrospectively in the serum collected on his first hospital admission but the gp 24 antigen was not detected in this specimen.

DISCUSSION

Erythema multiforme major in a 2-month-old child is rare. In our patient it is likely that this was drug-induced and probably related to the child having HIV virus infection. An increased incidence of adverse cutaneous reactions has been reported in patients with AIDS, particularly those treated with sulphonamides.[4–7] There are also reports of drug reactions to anti-tuberculous agents[8] and in one patient to various unrelated drugs.[9] A rash was observed in a 6-month-old girl with AIDS treated with co-trimoxazole,[10] but in a review of the cutaneous manifestations in children with human immunodeficiency virus infection only one case of EM is mentioned.[11] Severe bullous drug reactions in adult patients with AIDS have been described but only in association with sulphanomides.[12] An increased incidence of drug reactions in adult patients with AIDS makes it likely that in our case this retroviral infection was responsible for the development of EM, and the most likely culprit for the drug was phenobarbitone that is known to trigger off EM major[13] and TEN.[14]

A single pulse of methyl-prednisolone that produced rapid resolution of the lesions in our case is used for the treatment of inflammatory and autoimmune dermatoses,[15,16] but rarely for acute drug reactions.[17] We consider that this treatment is effective in arresting both EM major and TEN and producing a rapid clinical improvement.[18] However, there are acute and long-term complications of corticosteroid pulse therapy.[19]

In conclusion, erythema multiforme major is an extremely uncommon condition in the first 3 months of life. Conventional treatment with corticosteroids is not normally recommended in EM,[20] but in the condition of severe EM major pulse therapy with a high dose is effective. We consider in our case that infection with HIV-1 was a factor in producing the severe drug-induced eruption. Unfortunately, with the growing number of children with AIDS,[21] the incidence of these severe drug-induced rashes is likely to increase.

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Necrolytic migratory erythema: association with neuroendocrine tumour with predominant insulin secretion

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SUMMARY

We report a case of necrolytic migratory erythema in association with a neuroendocrine tumour producing predominantly insulin. As far as we know this has not been observed before.

CASE REPORT

An 80-year-old woman first presented in August 1988 with abdominal pain and constipation. Investigations revealed that she had a normochromic normocytic anaemia, and sigmoidoscopy at that time was normal and a subsequent barium enema showed only diverticular changes. In May 1989 she was seen with a desquamative rash and hepatomegaly but refused admission to hospital for further investigations.

In September 1989, she was admitted unconscious with low blood glucose and responded to intravenous dextrose. Subsequent history revealed that she had anorexia and weight loss. On examination, she was wasted with hepatomegaly. There were no focal neurological signs but she had a flat affect and performed poorly on mental scoring. The rash consisted of a figurate erythema with desquamation that was most marked in the perianal area and on the lower legs.

Investigations

Haemoglobin was 9·4 g/dl, MCV 87 fl, MCH 30·4 pg, ESR 35 mm/h. The vitamin B_{12} was 200 ng/l (normal range 150–800 ng/l), the serum folate 2·8 μg/l (normal range 3·5–15 μg/l) and the ferritin 77 ng/ml (normal range 30–300 ng/ml). Total serum protein was 47 g/l and the albumin 27 g/l. The serum zinc was 8·5 μmol/l (normal range 8–23 μmol/l). The following investigations were either negative or normal: clotting studies, immunoglobulins and protein electrophoresis, antinuclear factor, autoantibody screen, thyroxine and cortisol levels. Investigations at different times showed a blood glucose of 2·6 mmol/l, insulin 230 pmol/l, C-peptide 2690 pmol/l and

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proinsulin 171 pmol/l (normal range < 10 pmol/l). On another occasion blood glucose was 2·3 mmol/l, insulin 403 pmol/l and C-peptide 1802 pmol/l. Other investigations are shown in Table 1.

An abdominal ultrasound showed hepatomegaly with 'target' lesions suggestive of metastases and there were stones in the gall bladder. An abdominal CAT scan confirmed the above findings and showed an enlargement of the proximal part of the pancreas.

A liver biopsy guided with ultrasound showed on histology an infiltrating neoplasm with cords and islands of cells which had eccentric hyperchromatic nuclei and faintly granular eosinophilic cytoplasm with argyrophilic granules (Fig. 1). This was consistent with a metastatic neuroendocrine carcinoma. Immunostaining was negative to gastrin, glucagon and somatostatin, but showed a few positive cells with the insulin preparation. A skin biopsy showed a patchy perivascular infiltrate in the dermis and the upper epidermis showed an abrupt transmission to parakeratosis with superficial necrolysis. The lower part of the epidermis appeared normal. Immunofluorescence was normal apart from linear deposits of fibrin at the peaks of the dermal papillae.

Treatment

The patient was treated with an adequate nutrition and vitamin supplements. Dermovate NN ointment was applied locally to the affected areas which produced some improvement in her rash. In view of her age, the tumour was not treated aggressively but she received diazoxide to maintain an adequate blood glucose level.

Following her death, post-mortem examination confirmed the presence of a pancreatic tumour with metastases to regional lymph nodes and to the liver. Electron microscopy confirmed the neuroendocrine nature of the tumour with cells containing dense core granules (Fig. 2). These occasionally contained a crystalline body indicative of insulin secretion. Repeated immunostaining carried out on the liver and pancreas confirmed earlier findings.

DISCUSSION

Our patient was considered to have a malignant neuroendocrine tumour that secreted insulin, in view of the markedly raised fasting insulin together with the electron microscopic and immunostaining findings. The cause of her raised glucagon level is less certain as glucagonomas are usually associated with high levels between 1000–5000 pg/ml[1] and rarely below 500 pg/ml[2]

TABLE 1. Other investigations performed

	Fasting glucose (mmol/l)		
	3·4	7·4	Normal
VIP	4	5	< 30 pmol/l
Pancreatic polypeptide	525	845	< 300 pmol/l
Gastrin	6	4	< 40 pmol/l
Glucagon	374	131	< 50 pmol/l
Neurotensin	4	10	< 100 pmol/l

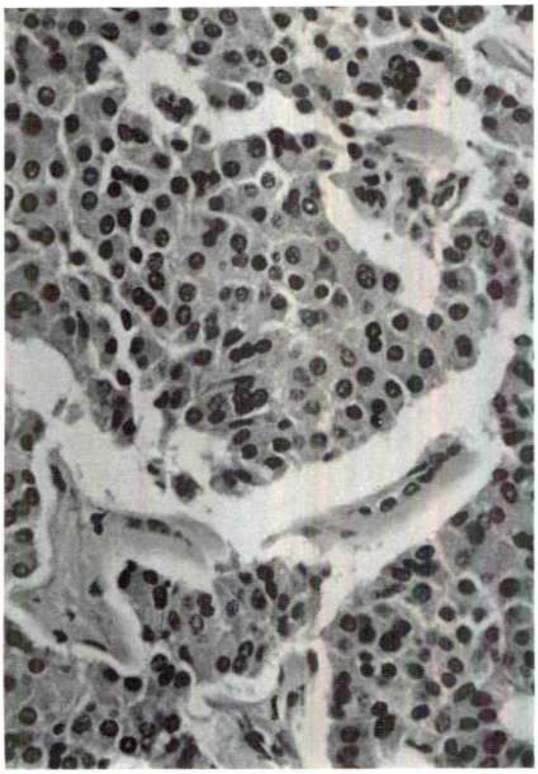

FIGURE 1. Liver biopsy showing a trabecular arrangement of uniform polygonal cells with round nuclei and granular cytoplasm. (Haematoxylin and eosin, × 80).

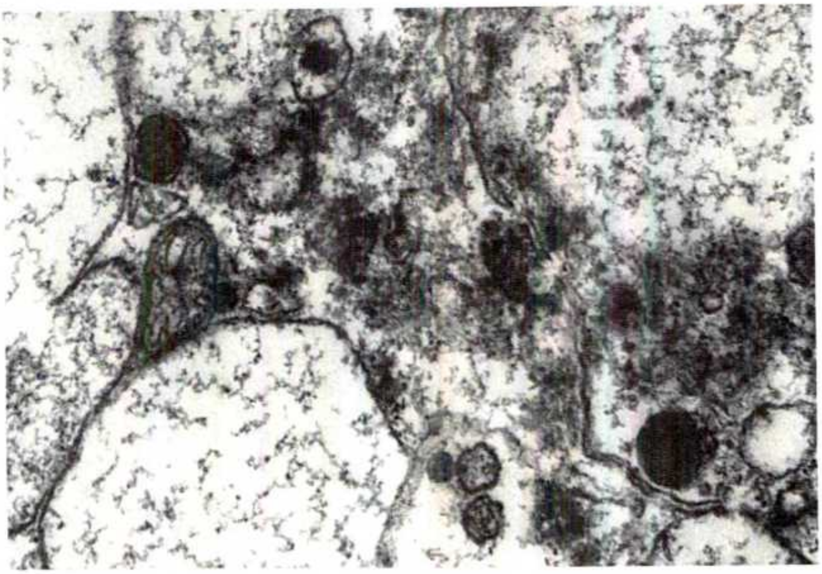

FIGURE 2. Electron-dense membrane-bound granules indicative of neuroendocrine origin; post-mortem tissue (× 39, 000).

(normal level < 200 pg/ml).[2] In a study of insulinomas, fasting glucagon was shown to be elevated when compared with controls.[3] This could be due to the secretion of glucagon by normal pancreas in response to hyperglycaemia or to secretion by the tumour. Evidence in favour of the latter comes from the finding of insulin staining within a glucagonoma[4] although in a previous study[3] glucagon levels were not different from controls when the patients were normoglycaemic.

In our patient, the failure of the glucagon level to suppress into the normal range with an adequate plasma glucose and the persistently raised pancreatic polypeptide suggest that this was a neuroendocrine tumour with a predominant but not exclusive insulin secretion.

The glucagonoma syndrome is caused by a tumour of pancreatic islet alpha cells[5] and consists of the association of necrolytic migratory erythema with weight loss, depression, stomatitis, venous thromboses, glucose intolerance and a normochromic normocytic anaemia. Our patient had several of these features. The syndrome has been previously reported without abnormal glucose tolerance,[6] but we are not aware of its occurrence with hypoglycaemia and a predominantly insulin-secreting tumour. Where it has been reported with multifunctional neuroendocrine tumours, they have been either predominantly glucagonoma[4] or insulinoma evolving into glucagonoma[7,8] with grossly elevated glucagon levels.

The rash in our patient was compatible both clinically and histologically with that of necrolytic migratory erythema. Skin biopsy shows spongiosis of the upper epidermis with necrolysis and parakeratosis. There is vacuolation of the cytoplasm with lysis of the intracellular organelles.[5,9] The eruption is associated with a raised plasma glucagon occurring in glucagonoma,[10] cirrhosis[11] and angioplasia.[12] Whilst therapeutic use of glucagon can produce a similar rash[13] it is not thought that glucagon itself is the cause of the rash as this can occur in chronic pancreatitis[14] and an ill-defined small bowel disorder[15] without a raised glucagon level. The histological similarities of the rash to pellagra and acrodermatitis enteropathica[16] suggest that it may be due to nutritional deficiency. Zinc, essential fatty acid and amino acid deficiencies have all been put forward as possible candidates. This might be the consequence of either a poor dietary intake, excessive utilization by the tumour or to a specific hormonal effect. The only relatively consistent abnormality is that of amino acids[17] and the low amino acid level resulting from the stimulation of hepatic gluconeogenesis by glucagon has been documented.[18] Correction of the amino acid deficiency does not always result in resolution of the rash[19] and there is a report of oral zinc consistently clearing the rash in a patient despite normal zinc and abnormal amino acid levels.[20] In our patient, improvement of the rash coincided with treatment with oral zinc and improvement in the blood glucose with the concomitant fall in her glucagon level. A rash has been described associated with a pancreatic polypeptide-producing tumour of the pancreas,[21] which was considered to be clinically and histologically different from that of a glucagonoma.

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Angiotropic B-cell lymphoma (malignant angioendotheliomatosis): failure of systemic chemotherapy

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SUMMARY

A 65-year-old female with angiotropic B-cell lymphoma is reported. Despite the absence of systemic involvement on formal staging and the favourable response of the cutaneous lesions to triple systemic chemotherapy with prednisolone, vincristine and cyclophosphamide, post-mortem findings showed that death was due to widespread disease dissemination.

Angiotropic B-cell lymphoma, a rare malignant disease, has recently been clearly separated from reactive angioendotheliomatosis and its origin identified as lymphoid rather than endothelial.[1,2] The condition has a predilection for cutaneous and neurological tissue but may infiltrate widely throughout the body. Death mainly occurs because of neurological involvement.[3] Treatment regimens employed in the past have included multiple systemic chemotherapy, surgery and radiotherapy.[4] The early use of a multiple chemotherapy regimen such as a combination of prednisolone, cyclophosphamide and vincristine[5] appears to be the most effective treatment[4] but due to the small number of patients with this condition it has been impractical to undertake controlled trials. We report a case where death occurred from disseminated disease despite marked improvement of the cutaneous lesions following systemic chemotherapy with prednisolone, cyclophosphamide and vincristine.

CASE REPORT

A 65-year-old Caucasian female presented with a 3-month history of severe burning pain on the inside of both thighs associated with enlarging indurated plaques (Fig. 1). The plaques showed a variable degree of erythema and telangiectasia and were painful and tender to the touch. Intermittent swelling of the lower legs especially on the left side had been reported and examination of the left shin showed some induration and telangiectasia. The patient was

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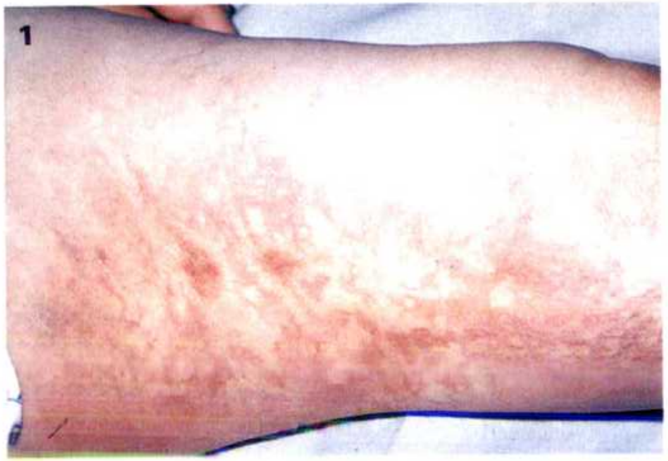

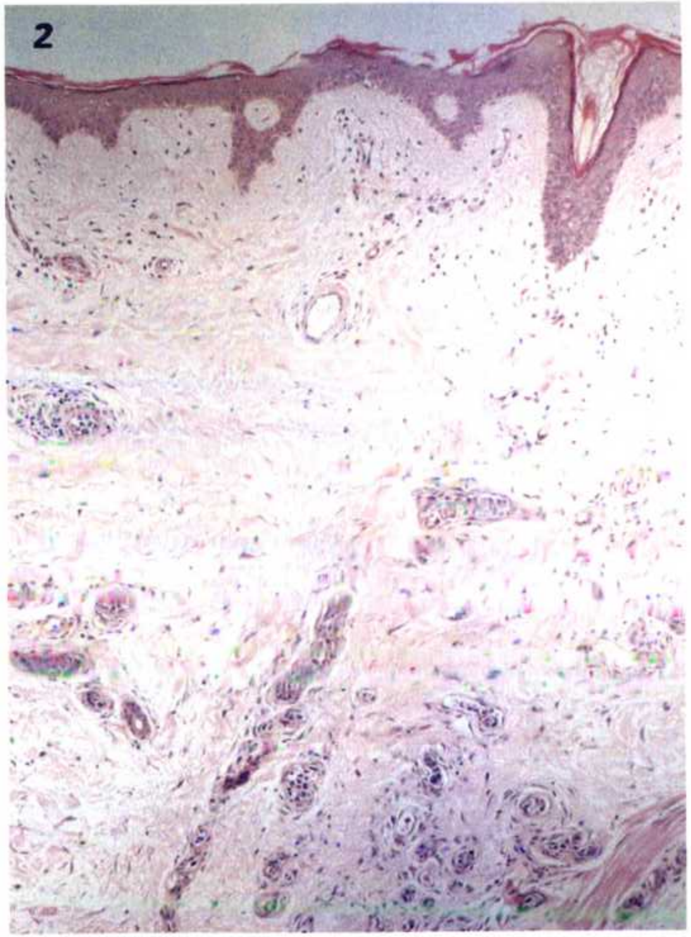

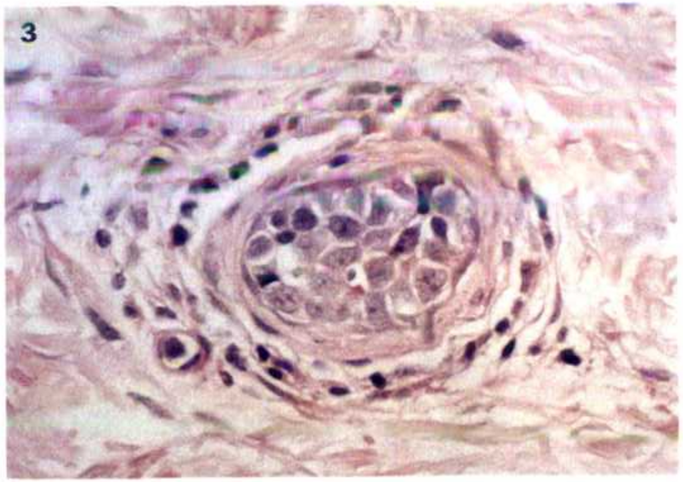

FIGURE 1. Close-up of thigh lesions showing telangiectasia against a background of indurated tissue.

FIGURE 2. Expansion of vascular lumina throughout dermis by malignant lymphoid cells. (Haematoxylin and eosin, × 28).

FIGURE 3. Angiotropic lymphoma. Lymphoid cells fill the lumen of this small blood vessel. (Haematoxylin and eosin, × 125).

generally well with no other abnormal physical findings and in particular no hepatomegaly or lymphadenopathy.

Histological examination of biopsies from both the thigh plaques and the lower leg showed widespread dilatation of capillaries, venules and small veins in the dermis and subcutaneous fat. The lumina of these vessels were filled with large pleomorphic mononuclear cells which did not appear to be in continuity with vascular endothelium but showed no evidence of extravascular spread (Figs 2 and 3). Immunohistological investigations showed these cells to stain strongly with anti-leucocyte common antigen (Dakopatts) and with the anti-human B-cell marker L 26 (Dakopatts). The cells did not express the CD1, CD3, CD4 or CD8 markers and were negative for Ki1 (Ber H2). In addition stains for factor VIII-related antigen and the epithelial markers CAM 5·2, CEA and EMA were negative. Attempts to demonstrate kappa or lambda light-chain restriction were technically unsuccessful. These features were fully consistent with a diagnosis of angiotropic B-cell lymphoma of the skin.

The results of initial investigations were haemoglobin 11·7 g/dl, white cell count $13{\cdot}3 \times 10^9$/l, lymphocytes $2{\cdot}87 \times 10^9$/l, platelets 510×10^9/l, erythrocyte sedimentation rate 87 mm in the first hour (Westergren) and a normal bone marrow examination. The following serum levels were all within normal ranges: urea and electrolytes, creatinine, calcium, phosphate, total protein, albumin, bilirubin, gamma glutamyl transferase, alanine aminotransferase, aspartate aminotransferase and urate. Serum alkaline phosphatase was marginally raised at 164 U/l (normal range 35–130). A staging laparotomy was not performed but a chest X-ray, abdominal ultrasound and CT scan of head, thorax and abdomen were all normal. Thus, at presentation, no evidence of extracutaneous spread was found.

Treatment

An initial oral course of chlorambucil 8 mg/day, prednisolone 40 mg/day and allopurinol 300/mg day, all for 7 days was accompanied by a marked expansion of the cutaneous lesions and deterioration in the accompanying symptoms. Four courses of more aggressive chemotherapy were then given over an 11-week period (courses 1–4). These consisted of cyclophosphamide 1 g i.v. and vincristine 2 mg i.v. administered on day 1 with prednisolone 60 mg being given daily for 7 days (5 days only in the case of course one). Prednisolone was also given continuously at the dose of 20 mg day from the end of course 3 onwards.

Chemotherapy was well tolerated but was associated with one episode of neutropenia and after course 4 by persistent thrombocytopenia with a platelet count of 12×10^9/l. During courses 1–4 there was progressive improvement in the appearance of the cutaneous plaques with a reduction in size and regression of induration. Following course 4 the burning pain in the patient's thighs had disappeared and only minimal thickening remained in the skin.

Two weeks following course 4 the patient became generally unwell with lack of energy and breathlessness on minimal exertion. Hepatomegaly was detectable. In spite of supportive in-patient management she died 3 weeks after course 4 with increasing dyspnoea, development of coma, persistent elevation of liver enzymes and deterioration in renal function. Her thigh skin remained almost normal with only minimal induration. During her terminal hospitalization chest X-ray, full pulmonary function tests (including measurements of transfer factor for CO and transfer coefficient), a ventilation perfusion scan of the lungs and abdominal ultrasound were all normal.

Post-mortem examination

Macroscopically the post-mortem examination showed no evidence of malignancy in any organ.

Moderate pulmonary oedema was present but there was no evidence of consolidation of the lungs. The liver was of normal size but the spleen was moderately enlarged (320 g) and had a firm uniformly red–brown appearance. There were no enlarged lymph nodes and no evidence of red marrow extension down the femoral shafts. On sectioning the brain there was a well established atheromatous infarct in the deep white matter of the occipital lobe. Histological examination showed widespread angiotropic lymphoma involving blood vessels in the lung, spleen, kidney and adrenals and diffusely permeating the liver sinusoids. Sections of skin from the original site of disease showed no evidence of residual lymphoma. There was no involvement of the bone marrow or of the cerebral blood vessels.

DISCUSSION

Treatments of angiotropic B-cell lymphoma have been of variable efficacy.[4] The combination of cyclophosphamide, vincristine and prednisolone, as used in this case, has previously been successful.[5]

Two points are worthy of special note in this patient. Firstly, death occurred because of widespread dissemination of angiotropic B-cell lymphoma despite the good clinical and histological response of the cutaneous lesions to systemic chemotherapy. Thus, the improvement of cutaneous lesions cannot be used as a judge of the overall systemic response to the chemotherapy regimen. Secondly, the extension of the disease beyond the skin was only recognizable on post-mortem histology; organ biopsy rather than imaging methods would have been required for ante-mortem diagnosis of dissemination and by implication for the monitoring of response to treatment in these extracutaneous sites. Interestingly, all of the patient's terminal signs and symptoms were explained by tumour infiltration of the relevant organ seen at post-mortem in spite of lack of evidence for this widespread infiltration ante-mortem using conventional imaging techniques.

A reliably efficacious chemotherapeutic regimen has not yet been identified for the treatment of angiotropic B-cell lymphoma. More aggressive chemotherapy regimens than that used in this patient may be indicated especially if initial histological staging demonstrates widespread systemic disease.

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Society Proceedings

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PAPERS

NIFEDIPINE MODULATES THE HAEMODYNAMICS AND VASOACTIVE REGULATION OF CYCLOSPORIN NEPHROTOXICITY IN THE NORMAL HUMAN KIDNEY. B.D.Edwards, [1]H.J.Testa, [1]R.S.Lawson, [2]R.J.Chalmers, [2]J.O.'Driscoll, F.W.Ballardie. Dept. of Renal Medicine, [1]Nuclear Medicine and [2]Dermatology, Royal Infirmary, Manchester, U.K.

Cyclosporin (CyA) induced dysfunction in the innervated normal kidney may result from disturbance of the microcirculation but its regulation, reversibility and means of abrogation are poorly defined. In other applications nifedipine may ameliorate CyA toxicity. Eight patients with psoriasis and stable cardiovascular state were treated with low dose CyA (2.5 mg/kg) for three months; after three months washout they then received an identical course of CyA with nifedipine S.R. 20mg b.d. The effect on GFR, RBF and filtration fraction (FF) was serially assessed using a novel Tc-99m DTPA dynamic technique(1) together with the measurement of the vasoactive hormones PRA, aldosterone, angiotensin II (A-II) and atrial natriuretic peptide (ANP).

Time (months)	(0)---CyA---(3)---No CyA---(6)---CyA+Nif---(9)			
GFR (ml/min/1.73m^2)	100 ± 6	85 ± 5^{a}	91 ± 4	87 ± 6
RBF (%C.O.)	17.0 ± 1.3	14.4 ± 1.3^{a}	15.4 ± 1.4	15.2 ± 1.2
FF (%)	23.6 ± 4.4	22.5 ± 2.7	21.7 ± 2.9	20.7 ± 1.53
PRA (pmoles/hr/ml)	4.4 ± 0.8	3.5 ± 0.4	4.0 ± 0.5	3.0 ± 0.4
Aldosterone(pmoles/l)	592 ± 57	411 ± 55^{a}	440 ± 54	427 ± 47
Mean AII (pg/ml)	8.7 ± 1.2	11.8 ± 1.7	8.8 ± 1.2	7.5 ± 0.8^{b}
ANP (pmoles/l)	3.3 ± 0.8	4.2 ± 0.3^{c}	2.9 ± 0.3	4.1 ± 0.4^{c}

Means ± SEM $p = <0.05$ ^{a}(0) vs.(3); ^{b}(3) vs.(9); ^{c}(3) or (9) vs.(6) month assessments

CyA nephrotoxicity was characterised by reduced GFR, RBF and hypoaldosteronism with a rise in ANP. Toxicity was reversible and did not recur after administration of nifedipine. A-II and FF were closely correlated ($r_p=0.46$; $p<0.02$).

CyA nephrotoxicity of the intact normal kidney may reflect A-II dependent efferent arteriolar vasoconstriction . These haemodynamic effects were abrogated in individual patients by nifedipine which may potentially be related to the modulation of A-II as well as calcium channel blockade. (1. Nucl. Med. Commun. 8: 823-37, 1987.)

"IRRITANTS" INCREASE THE CONTACT DERMATITIS RESPONSE TO ALLERGENS Janet McLelland and Sam Shuster, Dermatology Dept., Royal Victoria Infirmary, Newcastle upon Tyne.

Because we found that the contact dermatitis responses to allergens applied together were additive, we studied the effect of non-immune "irritant" inflammation on the response to allergen. 15 patients with a positive response to one of 7 allergens from the Standard European battery were studied. Serial dilutions of the allergen to which the patients reacted were applied on Finn chambers in duplicate to the upper arms. To minimise if not exclude an effect on allergen absorption the allergens were removed 24 h later. Serial dilutions of sodium lauryl sulphate (11 subjects) or anthralin (4 subjects) were then applied for a further 24 h to one set of allergen exposed sites, and, as a further control, to allergen-untreated sites. Responses were measured at 72 h as change in skin thickness measured by callipers and conventional clinical grading.

Size of response was directly related to the log dose of allergen or irritant applied. Responses were increased considerably at sites to which both allergen and "irritant" had been applied compared with either alone (slope of dose-response curve to both compared to allergen alone increased by 62%, $p<0.05$, ANOVA). Addition of "irritant" likewise lowered the threshold for a response to the allergens significantly from a mean of 2 to a mean of 4 dilutions ($p<0.05$, sign test, $n=8$). The clinical response gradings corresponded to those measured as change in thickness by callipers showing that "irritants" both augment the response to allergen and lower the threshold to them. It is concluded that irritants, even in subthreshold doses, increase the allergic contact dermatitis response by direct augmentation with reduction of the threshold of response. This, and the similar summation of antigen responses, explains the development of contact dermatitis in patients with negative patch tests to allergen alone. Likewise the "false-positive" patch tests of the "angry back syndrome" are nothing more than the lowered threshold of reaction of skin near an active eczema due to the summation we have demonstrated of the allergic and "irritant" response.

LACK OF EFFECT OF TERFENADINE ON ANTHRALIN INFLAMMATION E.L. Speight, B. Ramsay and C.M. Lawrence, Dermatology Dept., Royal Victoria Infirmary, Newcastle upon Tyne.

Anthralin inflammation is mediated by a free radical reaction with the production of active oxygen species. Since an active oxygen species will release histamine in vitro, we wondered whether this might be a component of anthralin inflammation. To test this we have studied the effect of the H1-receptor antagonist terfenadine on anthralin inflammation.

12 subjects (5 males, 7 females, mean age 36, age range 21-71) were randomised to receive terfenadine 60mg or placebo b.d. for 72 hours in a double blind cross-over study. The adequacy of histamine blockade was assessed using intradermal histamine, 8μg in 0.1ml normal saline on the second day of therapy. The weal area was measured planimetrically and weal thickness with Harpenden calipers. Weal volume was calculated by multiplying the weal area by half the increase in skin thickness. Dithranol 5, 10 and 20μg in 10μl chloroform was pipetted onto the flexor forearm skin. Anthralin inflammatory oedema and erythema were measured using Harpenden calipers and a reflectance photometer 48 h after anthralin application. After a 3 day washout period placebo and terfenadine were restarted and the same procedures repeated on the opposite arm. Results were analysed using two way analysis of variance.

One subject showed a small unexplained increase in weal area and thickness and was excluded from the study. In the other 11 subjects terfenadine treatment produced a mean reduction in weal volume of 91% (mean weal volume on placebo $361mm^3$, on terfenadine $32mm^3$). Erythema and oedema responses increased with anthralin dose, but the dose response curves on and off terfenadine showed no significant difference with and without terfenadine ($p>0.05$).

This study shows that H1-receptor blockade does not inhibit anthralin inflammation and suggests that histamine action on the H1-receptor does not have a significant role as a mediator of anthralin inflammation.

TOPICAL INDOMETHACIN AGGRAVATES THE WEALING AND FLARE RESPONSE IN CHRONIC DERMOGRAPHIC URTICARIA. G.R. Sharpe & Sam Shuster. Dermatology Dept., Univ. of Newcastle upon Tyne.

A topical preparation of indomethacin, sufficient to inhibit UVB erythema, was without effect on the weal and flare response to histamine, 48/80 and HDM antigen in normal subjects (Humphreys and Shuster, Br J Clin Pharmac 1990,**29**,195-199). It was therefore concluded that the worsening of chronic urticaria (CU) by aspirin-like drugs (cyclo-oxygenase inhibitors) was related to the urticarial disease process. To test this we have studied the effect of topical indomethacin on the wealing and flare response of chronic dermographic urticaria (CDU).

Nine patients (6 female, mean age 42 yrs) with CDU were studied; none had taken astemizole prior to the study or other antihistamines for at least 3 days. Two 25x6 cm rectangles of paraspinal back skin were used and a test dose of UVB radiation (40-65 mJ/cm^2), estimated to be 2-3x the minimal erythema dose, was given to a site on both sides. Indomethacin 1% gel was applied to one side and gel base to the other and the areas occluded. After 18 hrs the gel was removed and 30 mins later the erythema at the UVB test sites was measured with a reflectence meter. The increase in erythema over an unirradiated control site was 0.169±0.024 (mean±SEM, n=7) for the gel base and 0.094±0.018 for the indomethacin gel. The significant inhibition of UVB erythema by indomethacin ($p<0.002$, paired t test) confirmed the penetration and bioactivity of the indomethacin. The wealing response was tested using a spring loaded stylus traversed 3 times across a metal guide plate. The weal at 10 pressures (17.5-95.5 g/mm^2) was measured in triplicate and a pressure/weal response curve obtained by non-linear regression. The wealing threshold was 21.6±4.9 g/mm^2 (n=9) for the gel base and significantly reduced to 16.2±3.8 for indomethacin ($p=0.01$). The corresponding values for the maximum weal obtained were 5.4±0.56 mm and 5.7±0.88. A significantly greater degree of erythema around weals on the indomethacin side was observed in 6 patients.

We conclude that since indomethacin exacerbates the wealing of CDU that the wealing of this form of chronic urticaria is controlled by a non cyclo-oxygenase eicosenoid. This probably explains why CU and CDU may continue despite total histamine blockade.

EFFECT OF AN H1 ANTAGONIST (CETIRIZINE) AND H2 ANTAGONIST (RANITIDINE) ON THE SYMPTOMS AND WEALING RESPONSE IN CHRONIC DERMOGRAPHIC URTICARIA. G.R. Sharpe & Sam Shuster. Dept of Dermatology, Univ. of Newcastle upon Tyne.

This study was designed to investigate the efficacy of cetirizine, a new H_1 receptor antagonist, in chronic dermographic urticaria (CDU) and to see whether the addition of ranitidine, an H_2 antagonist, improved the response.

21 patients with CDU entered the trial and 19 (6 male, mean age 39 yrs) completed. After a 3 day run in period patients received either cetirizine 10mg daily and ranitidine 150mg twice daily or cetirizine and placebo indistinguishable from ranitidine, double blind. After a week of placebo (single blind) patients received the other treatment. Patients' kept a daily diary of itch and weal (100mm line) and sleep. Patients' mean assessment was: weal severity 40.8, 34.3, 16.8, and 13.8 mm for the run in, placebo, H_1 and H_1+H_2 periods respectively; itch 49.2, 43.8, 19.4, and 16.0 and % disturbed nights 47.0, 46.2, 8.8, and 9.1. 10 patients preferred the H_1+H_2 treatment, 7 the H_1 alone and 1 the placebo. The placebo effect was not significant; the treatment effects were significant ($p<0.01$) with no difference between the two.

At each visit a 10 point pressure/weal response curve was obtained with a spring loaded stylus traversed 3 times across a metal guide plate. The weal threshold and maximum weal was obtained by non-linear regression. The mean (SEM) weal thresholds for the run in, placebo, H_1 and H_1+H_2 periods were 24.9 (3.4), 26.1 (3.6), 55.6 (5.8) and 74.3 (4.6) g/mm^2. Both active weeks showed a highly significant increase in weal threshold ($p<0.00001$) and the small additional effect of the H_2 antagonist was significant ($p<0.001$). The mean maximum weals were similar for the run in, placebo and H_1 periods, 4.5, 4.7, & 4.1 mm, but not achieved on testing for the H_1+H_2 period.

The increase in weal threshold was similar to that previously found with terfenadine or astemizole combined with cimetidine. However the H_1 antagonist used both in the present and previous studies of H_2 antagonist achieved only partial H_1 blockade. Thus the small additional effect of an H_2 antagonist is probably due to further H_1 blockade which could be achieved equally by an increase in dose or potency.

CALCITONIN GENE-RELATED PEPTIDE AND ENDOTHELIN IN DIGITAL SKIN IN RAYNAUD'S PHENOMENON.

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The potent endogenous vasodilator calcitonin gene-related peptide (CGRP) is implicated in the control of peripheral cutaneous blood flow. Qualitative or quantitative digital CGRP mediated dysfunction may contribute to the pathophysiology of Raynaud's phenomenon (RP). The role of the endothelium-derived vasoconstrictor, endothelin (ET) in cutaneous microvascular control is not known. The distribution and quantity of CGRP and endothelin IR immunoreactivity (IR) in normal digital skin and that of patients with RP has been determined by immunocytochemistry.

Eight normal women (mean age 26.3, range 24-32 years), seven patients with primary RP (six women and one man, mean age 37.3, range 19-52 years) and eight patients with systemic sclerosis (SS) and RP (seven women and one man, mean age 58.4, range 45-80 years) were studied. An elliptical skin biopsy of the finger skin overlying the dorsum of a proximal phalanx was taken from each subject. Immunocytochemistry (IC) was performed after fixation in Zamboni's solution using specific rabbit polyclonal antisera for several peptides including the pan neuronal marker protein gene peptide (PGP), CGRP and ET. FITC conjugate and pontamine sky blue counterstaining were used. IR positive fibres in five adjacent x25 fields were counted blind by two observers and the scores were pooled and averaged.

All subjects showed a neuronal network of epidermal and dermal fibres (PGP IR) with no qualitative differences between the groups. There was a significant (Mann-Whitney U test: $p<0.05$) decrease in the number of CGRP IR fibres in the epidermis and papillary dermis of both patients with primary RP (median 8.8, interquartile range (IQR) 5.8-10.6 fibres/field) and SS (median 2.0, 1QR 1.6-4.0) compared with controls (median, 11.8 IQR 9.6-15.7). IR for ET was seen in small and large vessels (arterioles, capillaries and venules) in all layers of the dermis with no apparent differences between the groups.

These findings indicate a deficiency of CGRP nerves in RP, provide evidence for Lewis' local fault hypothesis and demonstrate for the first time the presence of endothelin in the cutaneous microvasculature.

TOPICAL STEROID AND THE VASCULAR RESPONSES TO INTRADERMAL NEUROPEPTIDES, HISTAMINE AND COMPOUND 48/80.

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The effect of steroids on the response to the pro-inflammatory neuropeptides Substance P (SP) and calcitonin gene-related peptide (CGRP) is not known. This is of interest because a) steroids are potent suppressors of inflammation in cutaneous diseases (e.g. atopic dermatitis) in which neuropeptides have been implicated and b) it allows further dissection of the mechanisms of action of neuropeptides in inflammation.

Eight volunteers (4 men and 4 women mean age 32, range 28-41 years) were investigated. At the beginning of the experiment a baseline evaluation was made in five subjects: the blood flow at five anteromedial forearm sites was recorded using laser Doppler flowmetry (LDF) at 20° C in a constant temperature and humidtiy (30%) Environmental Chamber. An intradermal injection of 25µl of either 10µg/ml histamine, 10µg/ml compound 48/80 (48/80), 1µM CGRP, 1µM SP or saline vehicle control was made at each randomly allocated site. After two mins. and after 30 mins. the blood flow was again recorded and the diameter of the visible flare was measured (mm) by planimetry. All subjects ($n=8$) then applied topical clobetasol propionate ointment three times a day and under occlusion at night to the anteromedial aspect of one forearm. Seventy two hours later the intradermal injections, blood flow measurements and planimetry were repeated on both the treated and untreated forearms.

There were no significant differences (Wilcoxon) between the LDF responses at either two or 30 mins. for any of the agonists either between treated and untreated arm when compared to baseline and treated arm when compared to untreated arm. The responses for flare showed significant ($p<0.05$) dimunition at two mins. for SP and 48/80 when the treated arm (SP:32.6+/-SD7.25; 48/80:33.7+/-SD4.82) was compared to the untreated arm (SP:40.7+/-SD11.8; 48/80:42.1+/-SD8.91), but showed no difference between the baseline evaluation and the untreated arm (SP:38.0+/-SD5.47; 48/80:43.2+/SD10.6).

These results show that LDF and flare are measuring different aspects of the vascular response. SP and 48/80 elicit mast cell histamine release and it is thought that it is this property which has been affected by steroid pretreatment.

HUMAN HAIR GROWTH IN VITRO.

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The factors that regulate cell division in the matrix cells of the hair follicle, and which control the hair growth cycle are poorly understood. Our lack of understanding of the regulation of hair growth has been caused, in part, by the lack of a good *in vitro* model. We now report on the successful growth of human hair *in vitro*, and on the *in vitro* effects of growth factors and mitogens on our model.

Human hair follicles were isolated from human scalp skin by microdissection. This was achieved by cutting the hair follicle at the dermo-subcutaneous fat interface using a scalpel blade; the intact hair follicle bulb was then removed from the fat without damage. Isolated follicles were maintained in individual wells of 24 well multiwells containing 500μl of supplemented Williams E culture medium; this permitted detailed measurements to be made on the length of individual follicles.

Length measurements made on these follicles in the presence of serum showed a significant increase in length over 5 days, the rate of increase being 0.3mm/day (n=6 samples, 36 follicles in total), and approximates to that seen *in vivo*. This increase in length was not associated with any disruption of hair follicle architecture and the increase in length was seen to be attributed to the production of a keratinised hair shaft. [methyl-^{3}H] thymidine autoradiography confirmed that the *in vivo* patterns of matrix cell DNA synthesis were maintained *in vitro*, and [^{35}S] methionine labelling of keratins showed that the patterns of keratin synthesis did not alter with maintenance.

It was observed that in serum free medium that hair follicle growth could be sustained for up to 10 days; this observation suggests that in serum free medium the hair follicles are able to regulate their own growth, possibly by the production of relevant growth factors. This should prove useful in identifying the autocrine / paracrine mechanisms that operate in the hair follicle. The importance of this model to hair follicle biology is further demonstrated by the *in vitro* observations that both EGF (10ngml) and TGF- (10ngml) resulted in the formation of a club hair like structure and also mimicked the *in vivo* depilatory action of EGF that has been shown in mice and sheep. We also report that TGF-ß1 (10ngml) was found to inhibit hair follicle growth *in vitro*.

BEARD AND SCALP DERMAL PAPILLA CELLS SECRETE SIMILAR MITOGENIC FACTORS BUT EXHIBIT DIFFERENT RESPONSES TO THEM

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Human hair follicles exhibit different responses to androgens. Since the mesenchyme-derived dermal papilla has a controlling influence on the otherwise epithelial hair follicle and cultured dermal papilla cells contain androgen receptors (Randall *et al*. (1989) J.Invest.Dermatol., 92, 503). We have investigated the secretion of mitogenic substances by cultured dermal papilla cells from androgen-sensitive beard and androgen independent scalp follicles and compared their response to such factors.

Conditioned medium (DP-CM) was prepared by incubating dermal papilla cells in serum-free medium E199 for 24h. After 48h in serum-free medium, cells (x3) were treated with various DP-CM for 24h before incubation with ^{3}H-thymidine (0.5 μCi/well) in fresh medium for 6h. Restriction of ^{3}H-thymidine to the nucleus was confirmed by autoradiography. Conditioned media (DP-CM) significantly increased ^{3}H-thymidine incorporation compared to control serum-free medium. There was no difference in DNA synthesis by dermal papilla cells (n=11) cultured in either beard (9 media) or scalp (8 media) DP-CM. However, whichever type of media was used beard cells (n=5) incorporated c.2x ^{3}H-thymidine than scalp cells (n=6). This was confirmed by treating 3 beard and 3 scalp lines with 15 identical media.

This demonstrates that both cultured scalp and beard dermal papilla cells secrete similar mitogenic factors, but their ability to respond is determined by their *in vivo* origin, i.e: they exhibit an altered gene expression *in vitro*. Such factors probably play an important role in human hair growth.

THE METABOLISM OF TESTOSTERONE BY CULTURED DERMAL PAPILLA CELLS DERIVED FROM HUMAN MALE BEARD FOLLICLES

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Human hair follicles exhibit different responses to androgens; beard growth is increased while balding may occur on certain areas of the scalp. In many androgen target-tissues the reduced metabolite of testosterone, 5α-dihydrotestosterone (DHT) is the active intracellular androgen. Since the mesenchyme-derived dermal papilla has a controlling influence on the otherwise epithelial hair follicle and cultured papilla cells have been shown to contain androgen receptors (Randall et al, 1989 J.Invest. Dermatol., 92, 503), we have examined the ability of dermal papilla cells cultured from male beard follicles to metabolise testosterone.

Confluent cells in 90mm petri dishes were incubated for 24h in serum-free medium, before incubation with 5nM $[1,2,6,7\text{-}^{3}H]$ testosterone for 2h. Control media with no cells were also assayed. The medium was removed and cells were washed with saline (x4). Both cells and media were extracted with chloroform:methanol (2:1). Unlabelled carriers and ^{14}C-marker steroids were added before separation by two different thin layer chromatographic systems; steroid identification was confirmed by recrystallization to constant $^{3}H/^{14}C$ ratio.

Approximately 80% of the radioactivity in the medium (n=4) was testosterone; the only metabolites identified were DHT (10%) and androstenedione (10%). Intracellularly (n=4), almost equal amounts of testosterone, DHT and androstenedione were recovered.

These results demonstrate the ability of cultured dermal papilla cells from human hair follicles to take up and metabolise testosterone. This is the first report of the intracellular metabolism of human beard dermal papilla cells in culture. They indicate that DHT is important in androgen-dependent hair growth, and concur the deficient beard growth of 5α-reductase lacking individuals.

ISOTRETINOIN TREATMENT ALTERS STEROID METABOLISM IN WOMEN WITH ACNE.

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A number of studies suggest that sebaceous gland inhibition by isotretinoin is not hormonally related but may cause secondary changes in the activity of skin steroid 5α-reductase. To obtain more information on the response of steroid 5α-reduction to treatment with isotretinoin we measured urinary steroid metabolites (µg/24 hr) by capillary column gas liquid chromatography and serum androgen levels (testosterone, androstenedione (A2), and dehydroepiandrosterone sulphate (DHAS)) by radioimmunoassay during the course of 16 weeks of therapy with isotretinoin (Roaccutane).

Seven women, median age 26.7 years (range 18 - 44) with persistent acne, non-responsive to antibiotics were studied. They had no evidence of hirsuitism, hair loss or virilism. All received 16 weeks of isotretinoin (1 mg/kg/day).

Acne grade fell from 3.5 (95% confidence interval, 0.3 - 6.6) to 0.25 (-0.05 - 0.5). Menstrual patterns did not change. Mean baseline concentrations of testosterone, A2, DHAS, and SHBG were normal. Over the 16 week treatment period there was a significant fall in serum testosterone at week 8 and 16 (p<0.05). Baseline total 24 hour excretion (µg/24 hr) of the selected steroid metabolites were within expected limits for normal women with significant falls of androsterone, tetrahydrocortisone (THE) and tetrahydrocortisol (THF) from week 8 onwards and aetiocholanolone and allo-THF from week 12 (p<0.05). Baseline ratios of both androsterone:aetiocholanolone (1.12 ± 0.09) and allo-THF:THF (0.89 ± 0.05) were at the upper limit of normal (normal mean values 0.65 ± 0.2, range 0.35 - 1.15 and 0.62 ± 0.2, range 0.2 - 1.0 respectively). These high ratios fell significantly during treatment suggesting that 5α-reductase activity is isotretinoin sensitive.

These results suggest a complex interaction between isotretinoin and steroid metabolism.

	baseline	**4wks**	**8wks**	**12wks**	**16wks**	**reference range**
Androsterone	1205±286	1098±226	596±121*	538±664*	664±194*	1029±457
Aetiocholanolone	1086±241	1175±288	730±157	609±207*	740±50*	1335±525
Tetrahydrocortisone	2708±527	2855±759	1480±234*	1553±322	1587±292*	3221±946
Tetrahydrocortisol	1331±238	1296±246	778±161*	835±214	750±133*	1617±525
Allo-tetrahydrocortisol	1293±302	1212±439	572±184	450±101*	548±117*	1008±491

(* = p<0.05, Wilcoxon Sign Rank test)

CICATRICIAL AND BULLOUS PEMPHIGOID: DOES THE COMPOSITION OF THE BMZ OF NORMAL SKIN, ORAL MUCOSA AND CONJUNCTIVA EXPLAIN THE CLINICAL DIFFERENCES?

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Bullous pemphigoid (BP) and cicatricial pemphigoid (CP) differ markedly in their clinical manifestations. The whole skin is involved in BP, whilst in CP the mucosae bear the brunt of the disease. Scarring occurs chiefly in the conjunctiva in CP. The aim of this study was to determine whether differences in the composition of the basement membrane zone (BMZ) of skin, oral mucosa and conjunctiva could explain these clinical observations.

Normal human skin, oral mucosa and conjunctiva were studied using patient's autoantibodies and monoclonal and polyclonal antibodies to BMZ antigens. A standard immunofluorescence technique was used. 6 BP sera (target antigen 220 kD), 4 CP sera (target antigen unknown), 2 pemphigoid gestationis (PG) sera (target antigen 180 kD), 2 epidermolysis bullosa acquisita (EBA) sera (target antigen type VII collagen 285 kD) and 8 linear IgA disease (LAD) sera (4 children, 4 adults) (target antigen 285 kD) were used to demonstrate the presence of their target antigens in the BMZ. The sera were titred to give a semi–quantitative measure of the relative amounts of antigen present. The monoclonal antibodies were the LH7.2 and L3d to type VII collagen, the hemidesmosome antibody G71, the dermal BMZ antibodies GB3 and LH39 and type IV collagen, and the polyclonal antibodies were laminin and fibronectin.

There was variation in the expression of these antigens between the different tissues. Skin expressed all the antigens best except type VII collagen and LAD antigen. Oral mucosa expressed type VII collagen and LAD antigen better than other sites, and BP and CP antigens less than skin or conjunctiva. Conjunctiva was relatively deficient in LAD and PG antigen, however BP and CP antigens were both well demonstrated. G71, GB3, LH39, type IV collagen, and laminin were demonstrated in all 3 tissues. Fibronectin expression at the BMZ was seen only in conjunctiva.

These findings suggest that it is not the relative concentrations of the antigens that determine the sites of predilection and the clinical picture of the diseases. In addition this study did not discriminate between the BP and CP antigens suggesting that they might be identical. The presence of fibronectin in the conjunctiva may be relevant to its propensity to scar.

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THE PATHOGENESIS OF HAILEY–HAILEY DISEASE: CLINICAL, IMMUNOPATHOLOGICAL AND TISSUE CULTURE STUDIES.

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We have investigated the pathogenesis of Hailey–Hailey disease using a suction blister technique, explant tissue culture and immunopathological staining with antibodies to the major desmosomal proteins, keratins, plasminogen and plasminogen activators. Patients were selected from 5 families with clinically and histologically typical Hailey–Hailey disease. In 3/3 suction blisters induced on the clinically normal forearm there was intraepidermal cleavage with acantholysis and fragmentation of desmosomes.

Immunopathological staining of skin biopsies from 5 lesions compared with 5 normal controls revealed normal expression of the major keratins, desmosomal proteins and plasminogen in perilesional skin. In lesional skin expression of "basal" keratins was no longer restricted to the cells of the basal layer but many of the suprabasal acantholytic cells continued to these keratins; there was internalisation of desmosomal components in both the acantholytic cells and the relatively intact blister roof and plasminogen was expressed not only by basal cells, as in normal skin, but also by the acantholytic cells.

The growth characteristics of explant tissue cultures of clinically normal abdominal skin from 6 patients were compared with explants from 10 normal controls. There was marked dissociation of the keratinocytes in the outgrowths of explants from Hailey–Hailey disease in comparison with the cohesive outgrowths seen in the controls. The major desmosomal proteins were present in the acantholytic cells but there was increased expression of both plasminogen and the plasminogen activator, urokinase, by these cells.

We conclude that in Hailey–Hailey disease there is a diffuse abnormality in cell adhesion, which may be revealed by mechanical stresses such as suction. Breakdown of the desmosomal–keratin filament complexes with secondary changes in the expression of keratins and desmosomal components may be initiated by increased activation of plasminogen.

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CONSERVATION OF A SUBUNIT OF BM-600 (GB3 ANTIGEN) IN HERLITZ SYNDROME - IMPLICATIONS FOR UNDERSTANDING PATHOGENESIS.

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BM-600, as recognized by the monoclonal antibody (Mab) GB3, is variably expressed in junctional epidermolysis bullosa (JEB) and its abnormal expression is used as a marker in the Herlitz form of JEB. Solid-support highly purified polyclonal antibodies (Pabs) have been raised against the three subunits of BM-600 and designated P α 100, Pα 125, P α 150. The aim of this study was to examine the expression of these subunits in skin samples from 19 patients with JEB (11 Herlitz, 8 non-lethal forms). Cryostat sections of each specimen were examined by two or three stage indirect immunofluorescence procedures using these Pabs as primary antibodies. Controls included normal human skin as a substrate (positive) and mouse serum in place of the primary antibody (negative). Samples from 10 Herlitz patients showed complete absence of GB3, P α 100 and P α 125 immunostaining, even after avidin-biotin amplification, but consistently positive P α 150 fluorescence. In the remaining 9 patients, the type of Pabs and GB3 immunostaining was similar in the same specimens, i.e. normal or reduced in intensity. These findings show that although BM-600 expression is absent or much reduced in the Herlitz form of JEB when examined by GB3 Mab, a subunit of the antigen identified by the Pab P α 150, is conserved and therefore possibly masked to GB3 binding. This suggests a partial defect of BM-600 and in order to determine whether this alteration is pre- or post-translational, in vitro-studies are continuing with cDNA probes.

RECOGNITION OF A STEM CELL GLYCOPROTEIN ON BASAL KERATINOCYTES.

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In this study we present evidence that a stem cell surface glycoprotein encoded by a gene on chromosome 11p is expressed by basal keratinocytes. The monoclonal antibody DF1516 recognises an antigen on bone marrow stem cells. In human-mouse hybridomas studies its expression depends on the presence of human chromosome 11. In immunocytochemistry experiments DF 1516 reactivity was found on the surface of basal keratinocytes but not other components of the skin. Epidermal cells suspensions were made by trypsin dissociation of separated epidermis. The suspensions were allowed to adhere to a collagen substratum. The collagen adherent cells were analysed for DF 1516 reactivity and 78-89% were found to be positive. The antibody detects a glycoprotein of 90-110 kDa in Western blots of epidermal extracts. The reactivity pattern and molecular weight data differ from those obtained from several growth factor receptors (EGF, transferrin receptor) and cell adhesion molecules (integrins, pemphigoid antigen, cadherins). These data suggest that DF 1516 recognises a differentiation molecule on basal keratinocytes and other stem cells but whose function at the moment is unknown.

PHOSPHOLIPASE A2 ACTIVITY IS INHIBITED IN INTACT LESION-FREE PSORIATIC EPIDERMIS BY THE NONAPEPTIDE HDMNKVLDL WHICH HAS LIPOCORTIN-LIKE ACTIVITY.

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Anti-inflammatory steroids are of value in the treatment of psoriasis probably because they induce the synthesis of the 40,000 Dalton protein lipocortin which inhibits epidermal phospholipase A2 (PLA2) leading to reduced concentrations of free arachidonic acid and inflammatory eicosanoids. A lipocortin-derived peptide is also a potent inhibitor of PLA2 in an epidermal homogenate (Cartwright et al. Brit. J. Dermatol. 1990, 122, 277-278). As part of a programme directed towards the development of an alternative to steroids in the topical treatment of psoriasis we now report the effect of the peptide HDMNKVLDL on PLA2 activity in an epidermal slice.

Superficial shave skin biopsies were obtained using a Davies dermatome and ethyl chloride local anaesthesia from the back in 6 patients with chronic plaque psoriasis. Skin specimens were obtained at least 5cm from the nearest lesion. Skin slices were incubated in Krebs Ringer bicarbonate containing glucose with or without the nonapeptide HDMNKVLDL at 5×10^{-8} M for 3 h. at 37°C. After rinsing in physiological saline, the epidermis was separated from the dermis, homogenised and the PLA2 activity determined as previously described (Cartwright et al. Brit. J. Dermatol. 1989, 121, 155-160).

For the control samples, the mean PLA2 activity ± S.E.M. was 111.2 ± 7.5 nmol/mg protein/hour ($n = 6$). For epidermal samples assayed after incubation with the peptide, the mean PLA2 activity was 82.5 ± 4.7 nmol/mg protein/hour ($n = 6$). This difference is statistically significant ($P < 0.01$, paired t test).

These results show that the nonapeptide HDMNKVLDL rapidly and significantly reduces the high PLA2 activity in intact psoriatic epidermis suggesting a therapeutic role for this agent when applied topically.

IDENTIFICATION OF A PLASMA KALLIKREIN-LIKE ENZYME IN HUMAN SKIN BLISTER FLUID.

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Skin diseases such as psoriasis and eczema are associated with itch and inflammation. Evidence exists that the kallikrein-kinin system may be activated in the skin of such patients (Hibino T, Izaki S, Kimura H, Izaki M, Kon SJ. Invest Dermatol 1988: 90: 505-10). Fluid withdrawn from suction blisters raised on the skin has been used to study the mediators present in normal and perilesional skin.

Double immunodiffusion tests were performed to establish the presence or absence of plasma kallikrein (PK) in blister fluids from patients with psoriasis, eczema, pompholyx and normal volunteers. Purified PK and normal plasma were used as positive controls. Enzymic activity of PK in blister fluid was measured on the selective, amidase substrate D-val-leu-argPNA. The enzymic measurements were made before and after activation of pre-PK by dextran sulphate.

Immunodiffusion tests showed that blister fluid from volunteers and all the patient groups contained immunoreactive PK. Studies on enzymic activity showed that 89-98% of amidase activity in blister fluid was inhibited by soya bean trypsin inhibitor, with a 2-3 fold increase in activity after dextran sulphate activation.

This is the first demonstration of a plasma kallikrein-like enzyme in human skin diseases. Activation of plasma and tissue (Poblete MT, Reynolds N, Figueroa CD, Burton J, Bhoola KD. J Physiol 1990; in press) kallikreins in epidermal inflammation and the consequent formation of kinins may be important in both the initiation of the inflammatory process and the pathological changes in the skin.

The study was approved by the Ethical Committee of the Bristol and Weston Health Authority, and we are grateful to the Psoriasis Association for financial support.

POTENTIAL ROLE FOR KERATINOCYTES AS INITIATORS OF CUTANEOUS INFLAMMATION. J.N.W.N.Barker, R.S.Mitra, C.E.M.Griffiths, V.M.Dixit, B.J.Nickoloff, Departments of Dermatology and Pathology, University of Michigan, Ann Arbor, U.S.A.

Infiltrating mononuclear cells accumulate within the skin in inflammatory cutaneous diseases such as psoriasis where they regulate via their production of cytokines, notably interferon-gamma (IFN-y) and tumour necrosis factor-alpha (TNF-a), keratinocyte elaboration of specific chemotactic factors and adhesion molecules for these same cell types. To examine whether keratinocytes may also be responsible for the initial recruitment of these cells into the skin, human keratinocytes were incubated with either all-trans-retinoic acid (RA) (1-10um), urushiol (catechol responsible for poison ivy dermatitis) (2um) and 12-0-decanoyl-13-phorbol acetate (TPA) (2-20nm). At varying times between 0 and 24 hours, RNA was extracted from the cell monolayers, electroblotted onto nylon membranes, and hybridised with ^{32}P-labelled cDNA probes specific for interleukin-8 (IL-8), IL-1b, TNF-a and intercellular adhesion molecule-1 (ICAM-1) mRNAs. Autoradiographic signals were quantitated by laser densitometry. IL-8, IL-1b and ICAM-I mRNAs were strongly induced by TPA, while there was weak induction of TNF-a. Urushiol upregulated IL-8, IL-lb and TNF-a strongly, and ICAM-1 weakly. RA induced IL-1b and ICAM-1 but not TNF-a or IL-8. In separate experiments, autocrine induction of TNF-a mRNA was noted in keratinocytes. These studies demonstrate that keratinocytes possess the immunological repertoire to respond to agents, known to cause cutaneous inflammation when applied topically, by producing mRNAs for specific cytokines and adhesion molecules. These findings potentially provide a molecular basis for the ability of a diverse array of environmental stimuli to produce cutaneous inflammation.

ANALYSIS OF STRATUM CORNEUM LIPIDS IN THE HEREDITARY ICHTHYOSES BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC). M.R. Judge, [1]M.S. Manku, [2]C. Stewart and J.I. Harper, Department of Dermatology, The Hospitals for Sick Children, Great Ormond Street, London, [1]Efamol Research Institute, Kentville, Nova Scotia, and [2]Scotia Pharmaceuticals, Guildford, Surrey.

Lipids comprise the major component of the stratum corneum intercellular matrix. Many of the hereditary Ichthyoses are associated with inborn errors of lipid metabolism and scaling is attributed to the altered lipid microenvironment.

A modification of the Folch method was used to extract stratum corneum lipids from skin scale of 75 patients with hereditary Ichthyoses. Topical treatment had been omitted prior to sampling. A one dimensional HPTLC method was used to separate all major stratum corneum lipid subclasses. 50µg of extracted lipid in 10µl chloroform:methanol was spotted on 60x60 prewashed silica plates and run in a two stage solvent system (chloroform methanol:water (95:20:1) followed by hexane:diethyl ether:acetic acid (80:20:10). The lipid bands were developed with 50% sulphuric acid and quantified in an LKB 2202 Ultroscan laser densitometer scanner. Standards were run on each plate and only non-treated (>3 weeks) scale samples were assessed.

As expected, there was a significant elevation of cholesterol sulphate (21% vs. 5%) in the 9 patients with X-linked ichthyosis compared with all other groups and a concomitant decrease in cholesterol (21% vs. 33%). Other notable findings included a high ceramide content in the 12 patients with bullous ichthyosis patients. Contrary to previous reports, free sterols exceeded ceramides in all groups except bullous ichthyosis and n-alkanes were elevated in all groups.

This technique, providing reliable analysis of scale lipids, will enhance our understanding of the abnormal lipid metabolism in the hereditary ichthyoses.

THE EFFECT OF TOPICAL SOLVENTS ON UV-B ERYTHEMA.

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It has been reported(1) that pre-treatment of the upper back and inner forearm of healthy and psoriatic volunteers with diethylether or 80% ethanol causes a significant increase in the UV-B minimal erythema dose compared with control sites, whilst cleaning after irradiation has no effect. That UV-B mediated erythema can be influenced by simply washing the skin with an organic solvent led us to investigate the validity of this observation using a reflectance instrument, rather than the eye, to quantify erythema.

Six columns, each comprising five 1cm diameter circles, were marked on the mid back of 13 volunteers. Three of the six columns, selected randomly, were wiped four times at each site with cotton wool soaked in a different solvent before UV-B irradiation and the remaining three columns were wiped immediately after irradiation. The solvents chosen were normal saline, 95% ethanol and diethylether. UV-B irradiation was given to each column using a fluorescent sunlamp with a geometric series of ultraviolet doses ranging from 75 to 198 mJ/cm^2. In all subjects erythema was measured at each of the 30 sites using a reflectance instrument both before irradiation and 24h after irradiation.

The increase in erythema index was plotted against the logarithm of the UV dose for each of the six columns on each subject. A Wilcoxon signed rank test for paired samples showed that for each solvent there was no statistically significant change in erythemal sensitivity between wiping the skin before or after irradiation. In no case did a solvent applied either before or after irradiation alter erythemal sensitivity of the group of subjects ($P > 0.2$; Kruskal-Wallis Test).

1. Barth J, Kadner H. Influence of topically applied organic solvents on UV-B minimal erythema dose (MED). Acta Derm Venereol Stockholm 1987: Suppl 134: 47-48.

ERYTHEMOMETRY OF LOW LEVEL CUTANEOUS HISTAMINE REACTIONS.

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The Lewis histamine injection response model is extensively used in cutaneous pharmacology. Existing methods for quantifying cutaneous weals and flares become imprecise at pathophysiological agonist concentrations; we have thus used electro-optical erythemometry as an alternative technique.

Saline placebo and histamine acid phosphate, dose range 1x10^{-9} to 1x10^{-12} (H9 to H12), volume 0.05ml, was intradermally injected into the forearm skin of 13 non-atopic volunteers. The Dia-Stron Model 1 erythema meter was used to measure reflected weal erythema index (WEI) [$\propto$ log 10(671nm)/(546nm)], range 0-999 arbitrary units (EIU) ±0.1%, at sites before injection and 10 and 20 minutes subsequently. WEI values and derived changes, absolute and relative, were normally distributed; Student's t-test (paired where appropriate) was used for analysis. Baseline WEI varied between individuals (mean 124.43 EIU; ± 2.71 SEM; range 61-201; n=130) but was uniform within subjects.

Although saline injection produced significant mean reduction from baseline WEI at 10 mins (-9.0 EIU, $p<0.02$), 5 sites showed a small increase (mean +6.6) and the largest was chosen as the threshold for positive histamine reaction. Using this criterion 29/104 (28%) injection sites were scored non reacting. Non-response rates were dose dependant over the range H9-H12 being 0%, 4%, 42% and 65% respectively. Using all data, correlation between WEI and log histamine concentration (t_{max} 10 mins) was apparent. Analysis of reacting sites alone revealed a clear dose response relationship at all tested histamine concentrations ($r=0.98$, $p<0.01$) and statistically highly significant ($p<0.001$) increases in WEI from baseline, H9: mean +50.24 EIU, H10: +43.36, H11: +27.73, H12: +17.89. Only data sets H9 vs H10 failed to differ significantly from each other, curve fitting suggesting a sinusoidal dose response linear over the range H10-H12 with approaching maxima at or near H9.

In 9 subjects flare erythema index (FEI) was also measured 7.5 mm distal to the injection point and outside the area of palpable wealing. Significant changes in FEI occured only at H9 and H10 but, importantly, mean incremental FEI significantly exceeded WEI at H9 (+ 70.78 vs +52.31, $p<0.001$) suggesting the onset of cutaneous weal zone vasoconstriction.

Cutaneous erythemometry at picomolar histamine concentrations is a new approach to the study of the Lewis response. It should also be of value in the study of novel vasoactive substances such as the neuropeptides.

MEASUREMENT OF CUTANEOUS INFLAMMATION USING A SCANNING LASER DOPPLER VELOCIMETER

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Although laser Doppler velocimetry (LDV) is used widely to measure skin inflammatory reactions, conventional instruments are limited by the small skin area that can be measured at one time. A scanning laser Doppler velocimeter (SLDV) has been developed that can measure blood flux at multiple sites in a short time period. We have compared its performance in the measurement of 4 skin inflammatory reactions with that of a conventional LDV instrument (Perimed Pf2).

UVB: 8 sites on the back were irradiated with a series of doses of UVB in 4 subjects.
Irritant reaction: 6 dilutions of sodium lauryl sulphate were applied for 48 h to forearm sites in 4 subjects.
Contact allergic hypersensitivity (CAH): 4 patients with known contact sensitivity were challenged with 7 different doses of antigen applied to the forearm for 48 h.
Histamine response: 3 doses of histamine were injected intradermally in 4 subjects.

UVB responses were measured at 24 h, and irritant and allergen at 72 h. SLDV measurements of histamine response were made at 10 min. For both instruments, increase in blood flux over background levels was used to construct dose response curves.

A significant ($p<0.01$) correlation between LDV and SLDV measurements was shown for UVB, irritant and CAH reactions. Detection threshold and coefficient of variation did not differ significantly for LDV and SLDV measurements. The SLDV also allowed area of inflammation to be measured (area of blood flux >2SD of background) which showed a significant correlation with log dose for UVB and CAH. Histamine flare was clearly defined using the SLDV, and flare area showed a linear increase with log dose ($p<0.05$).

SLDV is as sensitive as conventional LDV but has the considerable advantage of being able to measure blood flux over large areas without contact with the skin surface and can therefore be used to measure area as well as intensity of inflammation.

SUNSCREEN PROTECTION AGAINST UVB AND UVA: AN IN-VITRO AND IN-VIVO COMPARISON

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In-vivo measurement of UVA protection by sunscreens in normal skin is difficult because of the relative erythemal insensitivity of skin at this waveband. An in-vitro technique has recently been described which allows measurement of sunscreen protection at any wavelength (Diffey & Robson, J. Soc. Cosmet. Chem. 40:127,1989). Although sun protection factors calculated using this technique show good agreement with FDA and DIN reference formulations the technqiue has not been validated for UVA protection. We have used photosensitive patients to allow in-vivo measurement of UVA protection achieved by a widely prescribed sunscreen and a new product based on microfine titanium dioxide, and have compared the results with the in-vitro assay.

In-vivo protection factors were calculated from minimal erythema doses measured at 300, 350 and 400 nm in 11 patients with chronic actinic dermatitis before and after application (2µl/cm^2) of RoC 15+ A + B or microfine titanium dioxide (Crooks Health Care). In-vitro protection factors were calculated from measurements of spectral transmission at 300, 350 and 400 nm through Transpore tape with and without the sunscreens applied.

The in-vitro protection factors were in close agreement with those obtained in-vivo for all wavelengths. Both products showed high UVB protection, but the product containing microfine titanium dioxide showed significantly higher protection against UVA (350 and 400 nm) than RoC 15+ A + B ($p<0.01$; log rank test).

	Microfine titanium dioxide			RoC 15+ A + B		
	300 nm	**350 nm**	**400 nm**	**300 nm**	**350 nm**	**400 nm**
In vivo (median)	29	14	2.7	55	5.8	1.5
In vitro	24	14	4.2	49	6.2	3.0

The in-vitro technique allows accurate and rapid measurement of sunscreen UVA protection. Sunscreens based on microfine titanium dioxide should provide higher UVA protection than those currently available on prescription.

PUVA TREATMENT OF PSORIASIS COMPARING LAMPS WITH PEAK EMISSION AT 325, 352 OR 370 nm

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The lamps used in PUVA treatment emit a fluorescence spectrum from 320-400 nm, with a peak at 352 nm. This choice of lamp was not based on knowledge of the PUVA therapeutic action spectrum for psoriasis which remains unknown, but because it was the only high-intensity wide-area UVA lamp available when PUVA was introduced. In order to test the effectiveness of other lamps that are now available we have compared the response of psoriasis to PUVA using lamps with peak emission at 325, 352 & 370 nm.

24 patients with psoriasis involving both forearms were allocated randomly to treatment with 2 of the 3 lamps (one for each arm). Patients were phototested before the first treatment to ensure that equal erythemally-effective doses of UVR were given from each of the lamps. The forearms of each patient were treated simultaneously, 2 h after ingestion of 8-MOP (0.6 mg/kg). Treatment was given 3 times weekly for 6 weeks and the UVR dose increased by 40% each week. For each patient, the arm which showed more improvement was judged each week by a clinician unaware of the treatment given.

The median doses required for clinical clearance from each of the lamps were 32 J/cm^2 (325 nm), 129 J/cm^2 (352 nm) and 391 J/cm^2 (370 nm). Odds ratios showed a shorter time to clearance with the 325 nm lamps compared with the other 2 lamps ($p=0.014$), and an improved response when assessed weekly during the trial ($p<0.001$). There was no significant difference in response between the 352 and 370 nm lamps.

We have shown that UVA radiation <340 nm is more effective than longer wavelength UVA in PUVA treatment of psoriasis. Lamps with peak emission at 325 nm should be more effective than those used conventionally which have only 16% UV emission <340 nm. 325 nm lamps are also highly effective for UV phototherapy of psoriasis (Farr et al, BMJ 294:205,1986). Thus, a single unit containing 325 nm lamps could be used either for PUVA or UV phototherapy of psoriasis, avoiding duplication of irradiation apparatus.

THE RATE OF INCREASE BUT NOT THE THRESHOLD OF PUVA ERYTHEMA RESPONSE CORRESPONDS TO PLASMA PSORALEN LEVEL

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Although starting doses of UVA in PUVA treatment are often chosen according to skin type, the marked variation between patients in erythemal sensitivity to PUVA cannot be explained on the basis of skin type, or sensitivity to UVB radiation (Cox, Farr & Diffey, Arch.Dermatol. 125:1653,1989). Blood concentration of psoralen also shows considerable variation between patients. In order to study further the variation in PUVA sensitivity we have measured the blood psoralen concentration and PUVA erythemal response in 56 patients about to start PUVA treatment.

Oral 8-methoxypsoralen (8-MOP) was taken (0.6 mg/kg) 2 h before blood sampling and phototesting. Plasma psoralen concentration was measured by HPLC. Ten 1 cm diameter sites on the inner aspect of the forearm were exposed simultaneously to UVA (Philips TL09R lamps) with a dose range from 0.8 to 30 J/cm^2. Erythema was measured at 72 h using a reflectance instrument and the increase in erythema index over background levels was expressed as a logit function of log dose.

Plasma psoralen concentrations showed a log-normal distribution and values ranged from 20 to 819 ng/ml. Mean plasma psoralen concentration was significantly lower in patients taking 30 mg of 8-MOP than those taking a higher dose ($p<0.01$), but the mean concentrations were otherwise independent of dose. There was no correlation between plasma psoralen conclentration and minimal phototoxic dose, either estimated visually or calculated from the dose response curves. However, the slope at the mid-point of the dose response curve showed significant positive correlation with plasma psoralen concentration (Spearman rank correlation coefficient 0.53, $p<0.001$).

The variation in slope of erythemal response but not the variation in threshold sensitivity to PUVA can be explained by differences in plasma psoralen concentration.

CELL SURVIVAL AFTER UV-C TREATMENT OF FIBROBLASTS, T-LYMPHOCYTES IN G_0 AND DIVIDING LYMPHOCYTE LINES FROM NORMAL INDIVIDUALS AND AN EXCISION DEFECTIVE XERODERMA PIGMENTOSUM PATIENT.

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We have set out to establish the range of variation in the response of T-lymphocytes to the lethal effects of UV-C. A set of normal individuals was compared with an excision defective xeroderma pigmentosum patient (XP6BR). Both lymphocytes newly separated from whole blood (G_0) and T-lymphocyte lines were examined and compared with the responses of dermally derived fibroblasts.

The G_0 T-lymphocytes of normal individuals were more sensitive than fibroblasts (D_{10} lymphocytes $\sim$ 6 Jm^{-2}; D_{10} fibroblasts $\sim$ 15 Jm^{-2}) In the initial data set of 9 normal donors there appears to be more variation between G_0 T-lymphocytes from different donors (D_{10} donor 43 = 8.0 Jm^{-2} vs D_{10} donor 348 = 5.0 Jm^{-2}) than for fibroblasts. Repeat experiments on a single donor, 42 showed that one standard error was approximately 20% of any mean value for per cent survival. For XP6BR both G_0 T-lymphocytes and fibroblasts had very comparable responses (D_{10} $\sim$ 3.0 Jm^{-2}). Thus the distinction between the XP and normals is greater with fibroblasts than with G_0 T-lymphocytes.

Clonally derived dividing T-lymphocyte lines were established from two normal donors and XP6BR. For the normals the lines showed enhanced survival comparable with the response of fibroblasts. For XP6BR one showed a response identical with the G_0 cells the other proved slightly more sensitive (D_{10} $\sim$ 2.0 Jm^{-2}).

The UV-C resistance of lymphocyte lines from normal individuals compared with their parental G_0 cultures may lie in the differential ability of stimulated or unstimulated cells to excise pyrimidine dimers but might also reflect a direct effect of UV on the capacity of G_0 mononuclear cells to respond to mitogen. The lack of recovery of resistance in lymphocyte lines from the XP implies that there may be a component of repair in lymphocytes which is dependent upon DNA synthesis or proliferation which is absent in such cells.

ROLE OF DERMATOPHYTE PROTEINASES IN THE PATHOGENESIS OF FUNGAL INFECTIONS

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Dermatophyte proteinases ("keratinases") are produced by dermatophytes in vitro and may well play an important role in the pathogenesis of fungal infections in vivo. In order to determine the possible role of dermatophyte proteinases, their proteolytic activity has been measured in cultures at 1, 2 and 3 weeks post inoculation, using an assay method utilizing human stratum corneum as substrate. The study included cultures initiated from 35 patients (31M, 4F, 18-60 years of age), with infections of the groin area. 25 patients had T. rubrum, 6 E. floccosum, 3 T. violaceum and 1 T. tonsuran infection. PAS stained skin surface biopsies from the patients were examined for mean hyphal length (a function of density of infection), using a stereological technique. PAS stained histological sections of biopsies from the same patients were evaluated for the degree of eczematization, employing visual analogue scales.

At two weeks the mean proteolytic activity observed was T. rubrum (2131±1002), E. floccosum (1005.3±240.7), T. violaceum (520.0±75.3), expressed as µg/hr/ml Eqv.BSA. A positive correlation was found between the proteolytic activity and the degree of eczematization of the same patient ($r = 0.665$, $p<0.001$). There was no relationship between the duration of infection and the degree of eczematization ($r = -0.167$, p=NS). In skin surface biopsies from 15 of the above patients a positive correlation was found between the mean hyphal density and proteolytic activity ($r = 0.559$, $p=0.05$). No correlation was found between the mean hyphal density of these patients and the degree of eczematization ($r = 0.379$, p=NS).

The results of these studies suggest that the degree of expression of enzyme activity in vitro is related to the intensity of infection in terms of the amount of fungal elements present and the degree of host reaction. The expression of dermatophyte proteinases in vivo may therefore be an important factor in determining the pathogenesis of the infection.

VENTRAL NAIL CONTRIBUTION TO THE NAIL PLATE IS CONTINUOUS ALONG THE LENGTH OF THE NAIL BED

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We previously showed that about 20% of the final nail mass is produced by the nail bed distal to the lunula. The present study was done to see whether this formation of ventral nail was continuous along the nail bed, and to compare its rate with production of nail by the germinal matrix. Nail production was measured as increase in nail thickness, using a screw gauge micrometer, at 6 points defined by measurement along their length of 20 normal toe nails removed from 20 normal individuals following accidental injury. Thickness increased linearly over the lunula at a mean rate of 0.13mm/mm nail length, the absolute thickness increasing from 0.38mm/mm ± 0.02 at the beginning to 0.68mm/mm ± 0.03 at the centre to 1.23mm/mm ± 0.04 at its distal end. Thereafter nail thickness continued to increase linearly along the whole length of nail bed at a mean rate of 0.027mm/mm length, with a mean thickness for the mid point of 1.23mm/mm ± 0.04 & 1.39mm/mm ± 0.05 at the end of the nail bed, corresponding to a increase of 21% over the length of the nail bed from the end of the lunula to the onychodermal band. There was a mean decrease in thickness of 32% from the end of the nail bed to the distal free edge of the nail, presumably due to frictional wear. We conclude that nail growth is rapid and linear over the germinal matrix (lunula) after which nail continues to be formed over the whole of the nail bed at a constant, albeit slower, rate. This novel finding is important for the understanding of nail growth and disease, and especially for the kinetics of drug movement into nails, since continuous production of ventral nail provides a large and previously unsuspected route of rapid access for drugs into the nails, operating along the whole of their length.

SHORT DURATION TERBINAFINE THERAPY PENETRATES DISEASED DISTAL NAIL VIA THE VENTRAL NAILBED AND IS EFFECTIVE IN ONYCHOMYCOSIS.

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To test the hypothesis that, by forming a barrier to fungal invasion in growing nail, short duration therapy of onychomycosis is an effective treatment, 8 subjects with Trichophyton rubrum nail infection were treated with the fungicidal allylamine terbinafine (125 mg b.d.) for 14 days. The response was observed monthly for 6 months and was studied in detail in one affected nail in each subject. At each visit, a permanent record was made for study by marking the edge of clinically affected nail in pencil and the nailfold in ink; the marks were then transferred to paper with transparent cellophane tape. The distance from the proximal nailfold to the most proximal affected segment was measured with a micrometer. Nail growth rate was similarly measured by the progress of a score made in the nail over the lunula. Distal clippings from the study nail were taken for mycological examination and culture at each visit. All but one patient showed clear improvement, and 20 of 25 fingernails and 23 of 62 toenails initially affected were clinically completely cleared at 6 months. The onset of response, indicated by outward movement of the affected segments in the study nails, was observed as early as 4 weeks. The maximum rate of clearance of clinical infection corresponded to the nail growth rate (mean difference -1 ± sd 31 μ/day). Cultures from distal nail clippings were negative from 4 weeks in 7/8 subjects, although fungal hyphae were demonstrated for up to 6 months despite continuing response. In 2 cases there was late clinical relapse, associated with a reversion to positive mycological cultures. We conclude that as predicted short duration therapy is possible in fungal nail infections. However, the response occurred before a fungicidal barrier could have grown out. It is unlikely that the drug has diffused distally from the proximal nail plate over the matrix. Hence the ventral nail, which contributes up to 20% to nail mass as it grows along the bed, must provide rapid access of drugs to the site of distal disease.

INHIBITION OF HUMAN LYMPHOCYTE BLASTOGENESIS BY A FACTOR DERIVED FROM TRICHOPHYTON RUBRUM.

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Immunomodulation of the host response by the invading organism is a recognised feature of some fungal infections. This mechanism has recently been proposed to account for the chronic course of some human dermatophytoses, in particular, those caused by the anthropophilic fungus *Trichophyton rubrum*. A report that serum from a patient with deep (subcutaneous) *T. rubrum* infection depressed lymphocyte transformation to *T. rubrum* cytoplasmic antigen (C.A.), led to suggestions of a circulating inhibitor acting as an antigen specific T cell receptor blocker. To date this observation has not been further investigated.

We have used ^{3}H-thymidine uptake to measure lymphocytic proliferation to varying concentrations of common antigens; PPD, SKSD, C. albicans and *T. rubrum* C.A. in normal controls (n = 8) and in patients with chronic *T. rubrum* infection (n = 9). The mitogen PHA was used as a positive control in each experiment.

There was no significant difference in proliferative response between normal control and patient lymphocytes to any antigen tested. However complete inhibition of lymphocyte blastogenesis was found to occur when cells were incubated at a concentration of *T. rubrum*. above 10ug/ml. This concentration of *T. rubrum* C.A. also inhibited lymphocyte blastogenesis to the mitogen PHA but was not associated with loss of viability.

Although this concentration of circulating cytoplasmic antigen will only rarely be found in patients' serum, we suggest that this may occur in the microenvironment of the dermis and might account for the observed anergy to trichophytin in patients chronically infected with *T.rubrum*.

EXPRESSION OF MILK PROTEIN GENES IN THE SEBACEOUS GLANDS OF TRANSGENIC AND NORMAL MICE DURING LACTATION.

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In order to study the regulation of milk protein gene expression we have produced transgenic mice carrying the entire guinea-pig alpha-lactalbumin milk protein gene. These mice regulated expression of the transgene correctly in their mammary glands during lactation. Whilst no or negligible transcription was detectable in all other tissues tested, high levels of guinea-pig alpha-lactalbumin transcripts were found in the skin of lactating transgenic mice. In situ hybridisation analysis showed that expression was localised to the undifferentiated basal layer cells of sebaceous glands.

Further studies revealed high levels of endogenous beta-casein mRNA in normal lactating mouse skin, demonstrating that the transcription of milk protein genes in lactating mouse skin is a normal event, and was not peculiar to the transgene.

This finding highlights the developmental relationship of the mammary gland to other specialised structures of the skin, supports a role for epithelial-extracellular matrix interactions in the regulation of milk protein gene expression in vivo, and identifies the skin as a particularly accessible model system in which to study the regulation of milk protein gene expression. In addition, regulatory sequences taken from the guinea-pig alpha-lactalbumin gene may provide a means of directing heterologous gene expression to the sebaceous glands in transgenic animals.

THE EXPRESSION OF RAR-α, RAR-β AND RAR-γ IN HUMAN KERATINOCYTES AND DERMAL FIBROBLASTS IN VITRO

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Three classes of retinoic acid receptor, members of the family of steroid/thyroid hormone transcriptional activators, have been identified in the last couple of years and these presumably mediate the biological effects of retinoic acid. Previous results (Rees & Redfern, Biochem. J. 259:917-919,1989) have suggested that primary dermal fibroblast cultures may differ markedly in the expression of RARs. In the present experiments, we have examined RAR expression in fibroblast and keratinocyte cultures established from different patients and body sites. Primary dermal fibroblast lines were established from foreskin, fetal skin and from a variety of sites from patients of age up to 89 yrs. Keratinocyte lines were derived from fetal skin, foreskin or retro-auricular skin. Northern blots of RNA, extracted by homogenisation in guanidinium thiocyanate and purified on CsCl, were probed with human RAR-α, RAR-β and RAR-γ probes. All three RAR types were expressed in dermal fibroblasts but there was some variation between lines: in some fibroblast lines RAR-β mRNA was only barely detectable but clearly expressed in others. In one of nine fibroblast lines RAR-β was expressed at a very high level. There was some correlation between the expression of RAR-α and RAR-γ but no apparent corrrelation of RAR-β with the other RARs. RAR-β, but not RAR-α or RAR-γ, was induced in response to RA in the one fibroblast line tested. All keratinocyte lines expressed RAR-α and RAR-γ but the expression of RAR-β was undetectable: there was no apparent change in RAR expression after treating keratinocytes with RA, or after calcium shift.

RETINOIC ACID RECEPTOR EXPRESSION IN S91 MELANOMA CELLS

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Despite the recent rapid advances in the identification and analysis of retinoic acid receptors (RAR), the function of these receptors in relation to cell proliferation and differentiation is still obscure. S91 melanoma cells respond to retinoic acid (RA) by a reduction in proliferation and an increase in pigmentation and we are using this cell line as a model system for understanding the cellular roles of RA and RARs. The expression of RAR in relation to RA-treatment has been investigated by Northern blotting studies of RNA extracted from S91 cells. Treating S91 melanoma cells with either all trans or 13-cis-retinoic acid (RA) results in a rapid, dose-dependent induction of RAR-β. The induction of RAR-β, detectable within two hours, is not inhibited by cycloheximide and this suggests that RA directly induces transcription of RAR-β. Induction of RAR-β is detectable with 10^{-9} M all trans-RA, but 13 cis-RA is less effective, inducing a detectable increase in RAR-β expression at 10^{-8} M. Unlike F9 teratocarcinoma cells where the RA-mediated induction of RAR-β is inhibited by cAMP, there was no effect of cAMP or phosphodiesterase inhibitors on the induction of expression of RAR-β in S91 cells. The expression of RAR-α and RAR-γ transcripts, expressed at a low level in S91 cells, is apparently unaffected by retinoic acid.

MSH INCREASES INTRACELLULAR CALCIUM IN MELANOMA CELLS

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Evidence has been accumulating that MSH-stimulated cyclic AMP production is not the sole or adequate stimulus for melanogenesis in some melanoma cells (Hill et al. 1989 Pigment Cell Research 2 161-66) and human melanocytes (Friedmann et al Brit.J.Derm. In Press). Indirect pharmacological evidence indicates that calcium (Buffey et al. Brit.J.Derm. 1990 Abstract) and diacylglycerol (Friedmann et al 1990 J.Cell Physiol. **142** 334-341) may also be involved in the control of pigmentation. Our data would suggest an inhibitory control of melanogenesis by both calcium and calmodulin (Mac Neil et al 1984 Biochem.J. 224 453-460, Mac Neil et al 1984 J.Invest. Dermatol. 83 15-19). Accordingly in this study we examined for the first time the direct effect of MSH on intracellular free calcium in a pigmented cell.

Murine B16 F1 melanoma cells were cultured as subconfluent monolayers on glass coverslips, loaded with Fura 2 AM for 1hr at 37°C, washed free of unloaded dye and their fluorescence at 490 nm examined with an excitation wavelength of 340nm. 71% of monolayers of cells examined showed a change in basal free calcium ($[Ca^{2+}]i$) in response to MSH. The predominant response in 46% of the responding coverslips was a delayed increase in $[Ca^{2+}]i$. 57% of monolayers showed an increase in $[Ca^{2+}]i$ to very low concentrations of MSH (10^{-12}M MSH). The average time delay in responding to MSH was 2.5 min at 10^{-12}M MSH, decreasing to 1 min at 10^{-8}M MSH. Addition of MSH increased $[Ca]^{2+}i$ from 230 ± 55nM to 540 ± 103nM (mean ± SEM, n = 6).

The ability of MSH to increase $[Ca^{2+}]i$ is the first such report to the best of our knowledge. These results raise the possibility that MSH may interact with receptors linked to two separate enzyme systems and that the melanogenic response to MSH involves the co-ordinated action of both cyclic AMP and calcium.

PENTOXIFYLLINE ENHANCES LUNG COLONIZATION AND INHIBITS GLYCOSAMINO_GLYCAN SYNTHESIS BY METASTATIC MELANOMA CELLS.

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Pentoxifylline (Trental), initially used to increase peripheral blood flow, and now shown to enhance the lethality of alkylating agents in human cancer cells, has recently been reported to inhibit extracellular matrix synthesis and enhance collagenase activity in fibroblast cultures. As extracellular matrix molecules and collagenases play important roles in the complex steps of metastatic spread of tumour cells, we have investigated the effect of pentoxifylline on metastatic melanoma cell lung colonization and glycosaminoglycan (GAG) synthesis.

B16F1M2 cells were derived from the B16F1 cell line by successive passages through the lungs of syngeneic mice. Cells were grown in Eagles MEM containing 10% foetal calf serum for 4 days in the presence or absence of 250µg/ml pentoxifylline. Lung colonization was assessed by injecting 10^5 cells via the tail vein of syngeneic mice, and counting lung tumours 18 days later. GAG synthesis was examined by isolating ^{3}H-glucosamine and $^{35}SO_4$-labelled GAGs by ion-exchange chromatography, and characterised by their susceptibility to degradation by specific enzymes.

Pentoxifylline inhibits cell growth, cell numbers being reduced by 31% following 4 days incubation, while the treated cells appeared more flattened, possessed numerous but short dendritic processes, and exhibited greatly enhanced melanin synthesis. Pentoxifylline treatment increased the cells ability to colonize the lungs, lung tumours increasing from 18.4 ± 6.15 to 61.5 ± 15.8, which was highly significant, $p<0.01$. In addition, synthesis of glycosaminoglycans was reduced by 36% following treatment with pentoxifylline, while the charge density of chondroitin sulphate was also reduced.

The observed changes in GAG synthesis may contribute to the cells increased ability to colonize the lungs, but changes in collagenase activity may also be important. As pentoxyifylline increases membrane flexibility, the resultant increase in cell deformability may also contribute to enhanced lung colonization.

ENDOTHELIAL LEUKOCYTE ADHESION MOLECULE-1 EXPRESSION IN ECZEMA AND PSORIASIS: A QUANTITATIVE STUDY.

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Expression of adhesion molecules by endothelial cells is likely to be of key importance in the recruitment of inflammatory cells at sites of cutaneous inflammation. We have examined and quantified immunohistochemically the expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), a transiently inducible endothelial molecule with adhesive properties for neutrophils, in normal skin (n=8), psoriasis (n=6, both lesional and non-lesional) and chronic eczema (n=5). Sections were stained with a monoclonal antibody to ELAM-1 and an endothelial marker, EN4. Labelling was quantified by counting 4 adjacent high power fields in the superficial dermis.

In normal skin a few vessels were faintly ELAM-1 positive (mean=8/4hpf). There was no significant difference between this and non-lesional psoriatic skin (mean=6/4hpf). In lesional psoriatic skin, many strongly labelled vessels were apparant (mean=32/4hpf) and this was significantly different from non-lesional psoriatic skin (p=0.009). In chronic eczema also, ELAM-1 expression was high (mean=23/4hpf). Although more vessels were present in the lesional psoriatic and eczema samples as assessed by EN4 staining, this did not account for the increased ELAM-1 expression noted as the percentage of ELAM-1 positive vessels was raised in both lesional psoriatic (55%) and lesional eczema (34%) skin when compared with non lesional skin (15%).

These results suggest that ELAM-1 expression is raised in two dermatoses of differing aetiology. The highest expression occured in psoriasis and may be of relevance to the neutrophilic nature of this disorder. However our results do not suggest a basal abnormality of ELAM-1 expression in this disease.

CD4+/CD45 RO+ HELPER INDUCER LYMPHOCYTES IN SKIN - IN VIVO EVIDENCE OF SELECTIVE UPTAKE.

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CD4+ lymphocytes have recently been divided into two immunoregulatory populations: helper inducer (THi) (CD4+/CD45 RO+/CD45 RA-) and suppressor inducer (TSi) (CD4+/CD45 RO-/CD45 RA+) lymphocytes. These populations may represent distinct cell lineages with differing effector roles or may be maturational stages of the same populations.

We developed a novel double immunohistochemical staining technique which enables two separate antigens to be co-localised to an individual lymphocyte cell surface - a procedure necessary for in situ demonstration of THi and TSi lymphocytes at sites of cutaneous inflammation. In both inflammatory dermatoses and around epidermal tumours, THi lymphocytes formed the great majority of the inducer subset populations, with very few TSi cells seen. This contrasts with peripheral blood where the two populations are present in approximately equal numbers.

To investigate the mechanism for THi predominance in skin suction blisters were raised over the sites of positive Mantoux reactions at 12, 24, 48 and 96 hours and analyzed for inducer populations using FACS. These sequential studies strongly suggest a selective uptake of THi from the circulation, with no evidence of early recruitment of TSi seen. These findings argue against phenotypic conversion of inducer lymphocytes occurring in skin.

POSTERS

CARRIAGE, SEROLOGY AND IMMUNOLOGICAL RESPONSES TO *MALASSEZIA FURFUR* SEROVARS A,B AND C.

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Malassezia furfur has been implicated as the aetiological agent of both pityriasis versicolor and seborrhoeic eczema. The aim of this study was to assess the carriage of *M.furfur* in 6 normal subjects and to determine their immunological responses to the three serovars of *M.furfur* recently defined (Cunningham et al., 1990, J.Appl.Bacteriol., in press) before studying patients with the two diseases.

Microbiological sampling was carried out in triplicate using the Williamson and Kligman Scrub technique, on the forehead, both cheeks, chest and back. Viable cell counts were obtained for *M.furfur, Propionibacteria* and *Micrococcaceae.* The *M.furfur* isolates were differentiated into serovars A,B and C and the subjects immunological status to each of them determined using lymphocyte transformations.

The population density for each group of organisms varied by upto 100 fold at the same site. This variation was most likely due to the distribution of follicles within the skin site. Isolates of *M.furfur* were serotyped and all 6 subjects carried all 3 serovars.

The immunological responses to *M.furfur* also displayed a large variation. 3 of the 6 subjects showed no sensitisation to the 3 serovars of *M.furfur*, although all 6 subjects were culture positive for the 3 serovars. Of those subjects who showed sensitisation to *M.furfur*, none were sensitised to all 3 serovars; 3 were sensitised to serovar B and 2 to C, but none showed any response to A. This suggested that serovars B and C were more accessible to the immune system than A.

CELLULAR LOCALIZATION OF TISSUE KALLIKREIN AND KININOGEN IN THE NORMAL AND PSORIATIC HUMAN SKIN.

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Tissue kallikrein (kinin-forming enzyme) has been partially purified from normal human sweat (Hibino T, Takemura T, Sato K. J Invest Dermatol 1988; 90: 569) and psoriatic scales (Hibino T, Izaki S, Kimura H, Izaki M, Kon S. J Invest Dermatol 1988; 90: 505-10), but the precise cell that contains the enzyme has not been identified. We have examined the cellular localization of tissue kallikrein and its substrate, kininogen in human skin biopsies obtained from both normal volunteers and psoriatic patients. Immunocytochemistry was performed using the peroxidase-anti-peroxidase and immunogold-silver staining methods.

Immunoreactivity to both enzyme and substrate was observed in secretory granules of the dark cells located in the secretory fundus (acinus) of sweat glands. When double immunostaining was performed on the same sections a segmental distribution of the two antigens was revealed. Each acinar section contained either tissue kallikrein or kininogen, but there appeared to be a functional zone in which both were present but in separate dark cells. Immunoreactivity for both antigens was also observed in close apposition to the luminal microvilli of the sweat duct cells. In addition, the epidermis and scales of the psoriatic skin contained many neutrophils immunoreactive for tissue kallikrein. Kinins may account in part for the increased mitotic activity seen in psoriatic epidermis and locally applied antagonists may prove of therapeutic value.

We are particularly grateful to the Psoriasis Association for financial support. Ethical Committee approval was obtained for this study.

LR4005 INHIBITS THE ω-HYDROXYLATION OF 12(S)-HETE BY PMNL'S.

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Incubations of peripheral blood PMNL's and platelets with arachidonic acid have been demonstrated to produce 12(S)-HETE and its ω-hydroxylation product 12,20-DiHETE. Using this system, increased production of 12,20-DiHETE was found in psoriatic patients (Maurice PDL et al. *Br. J. Dermatol.* 1986; **114**: 553-556). Whereas the addition of benoxaprofen to these incubations reduced the levels of 12,20-DiHETE in both psoriatics and normals, suggesting that the drug inhibits neutrophil ω-hydroxylation, indomethacin showed no such effect. Interestingly, dithranol has been demonstrated to inhibit the ω-hydroxylation of LTB_4 in vitro(Schroder J-M *J. Invest. Dermatol.*1986:**5**:624-629). Because BXP was an effective treatment for psoriasis, we have investigated the effects of a BXP analogue LR 4005 on the ω-hydroxylation of 12(S)-HETE to 12,20-DiHETE by PMNL's taken from the peripheral blood of normal subjects.

Standard techniques of lymphocyte separation using ficoll hypaque separating medium, dextran sedimentation and centrifugation, were used to separate PMNL's from the venous blood of 6 healthy volunteers. The subjects denied taking the contraceptive pill, aspirin in the preceding 14 days, or any other systemic therapy.

Aliquots of PMNL's containing 5×10^6 cells / ml in Dulbeccos buffer, were incubated with 12(S)-HETE (0.5 μg/ml) and various concentrations of LR4005 (0 to 20 μg/ml) for 10 minutes at 37 °C. The reaction was terminated by the addition of an equal volume of ice cold HPLC eluant, consisting of 75% methanol, 25% water, 0.06% acetic acid, pH 5.3. This mixture of cells and HPLC eluant was whirlimixed and then centrifuged at 13000 rpm on a microfuge for 5 minutes.

RP-HPLC was used to determine the concentration of 12(S)-HETE and 12,20-DiHETE in the supernatant. This was expressed in ng/ml. At a concentration of 5 μg/ml, LR4005 reduced neutrophil ω-hydroxylation of 12-HETE to 12,20-DiHETE by 50%.

It is suggested that the ability of a compound to inhibit neutrophil ω-hydroxylation might give an indication of its potential as a treatment for psoriasis.

CHARACTERISATION OF HISTAMINE RECEPTORS ON HUMAN ENDOTHELIAL CELLS

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Histamine induces release of vasodilator prostaglandins from human endothelial cells, a mechanisms thought to be part of its action in hypersensitivity and pro-inflammatory reactions. Our previous studies have indicated that prolonged exposure of human endothelial cells to histamine induces a biphasic release of prostacyclin (PGI_2) characterised by an initial period of rapid PGI_2 release followed by a period of slow sustained release. The aim of the present study is to characterise the sub-types of histamine receptors on endothelial cells which facilitate the biphasic response to histamine.

Confluent monolayers of first passage human umbilical vein endothelial cells (HUVEC) were used for these experiments. The HUVEC were incubated with the 'selective' H_1 receptor agonists 2-methyl histamine (2-Me), β-histine (β-his), and thiazolylethylamine (TEA), the H_2 agonists dimaprit and impromidine and the H_3 agonist R-α-methylhistamine (R-αMe). All of the H_1 agonists and the H_3 agonist R-αMe induced a concentration (10^{-7}M-10^{-3}M) and time dependent release of PGI_2 but were less potent than histamine itself. The rank order of potency was the same following 30 minutes and 24 hours incubation, i.e. histamine>TEA>2-Me> R-αMe>β-his (n=5) Neither dimaprit nor impromidine induced release of PGI_2 (n=2).

The H_1 antagonist mepyramine (10^{-5}M) abolished release of PGI_2 induced by histamine and the H_1 agonists. The H_2 antagonist cimetidine (10^{-5}M) reduced release of PGI_2 induced by 2-Me and β-his but this reduction was not significant. After 30 min. incubation, the H_3 antagonist, thioperimide (10^{-8}M-10^{-3}M) induced a concentration dependent release of PGI_2 which was comparable to that induced by β-his. No further increase occurred over 24 hours. Following 30 min. incubation, thioperimide (10^{-7}M-10^{-4}M) did not modulate histamine release of PGI_2 but after 24 hours incubation thioperimide (10^{-4}M) reduced histamine evoked release of PGI_2 by 35 ± 10%, n=3.

These results indicate that histamine induced release of PGI_2 from HUVEC is initiated primarily via H_1 receptor occupancy. The inhibitory action of cimetidine on H_1 agonist induced release of PGI_2 indicates that HUVEC also possibly possess H_2 receptors, occupancy of which augments the cellular responses initiated via H_1 receptor occupancy. The effects of the H_3 receptor agonist and antagonist indicate that there may be synthesis of histamine by HUVEC.

INTRAVITAL NATIVE AND FLUORESCENT ANGIOGRAPHY FOR THE ASSESSMENT OF THE SKIN MICROVASCULATURE: A PRELIMINARY REPORT.

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The study of the skin microvasculature in vivo could provide much information on the contribution of the microcirculation in the pathogenesis of skin diseases but at present it is limited to the study of nailfold capillaries which represent a special anatomical site (Bollinger A.,Frey J., Jäger K. et al. Patterns of Diffusion through Skin Capillaries in patients with long-term Diabetes. N.Engl.J.Med. 307: 1305-10 (1982)). This is a preliminary report of the study of non-nailfold skin microvasculature using intravital capillaroscopy.

Normal subjects and patients with psoriasis were studied in a temperature controlled laboratory. Tapestripped forearm skin was visualized with a Wild-Leitz through-lens light microscope attached to a video camera and recorder. The same group of capillaries were studied with native capillaroscopy, before and after venous occlusion, and with fluorescence angiography after the intravenous injection of 0.01 ml/kg body weight 20% Sodium fluorescein.

In normal skin the capillaries and subpapillary plexus were visualized, although surprisingly few capillaries were seen in the resting state. Venous occlusion increases the number of capillaries seen in any given area. Fluorescence angiography shows that the skin capillaries are intermittently perfused with a slow leakage of the tracer over several minutes forming a pericapillary halo. In contrast, in psoriatic skin the capillaries are easily seen, elongated and tortuous with an apparent greater density of blood vessels, both in lesional and perilesional skin. Fluorescence angiography reveals a greater perfusion of the capillary bed and rapid trans-capillary diffusion of the tracer.

This technique is a direct and non-invasive method for the assessment of intravital capillary morphology and blood flow dynamics both in the normal skin and in disease states. The technique allows sequential monitoring of the same capillary bed during the evolution of a disease and response to treatment.

TYROSINASE EXPRESSION IN A NEW AMELANOTIC SUBLINE OF THE B16 F10 MOUSE MELANOMA

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We have recently described a new amelanotic subline of the B16 F10 mouse melanoma. In order to understand why this subline, B16 F10DD, is less melanotic than B16 F10 we have examined its level of tyrosinase expression.

The F10DD cells contained less than 10% of the melanin found in F10 cells and this was reflected in their lower levels of tyrosinase activity. However, the subcellular distribution of tyrosinase activity was similar in the two sublines with approximately 80% associated with the melanosomal fraction. Tyrosinase synthesis as measured by immunoprecipitation of [^{35}S]methionine labelled proteins with tyrosinase antibodies, was also similar in the two sublines as were the levels of tyrosinase mRNA as revealed by Northern blot analysis using a [^{32}P] labelled cDNA insert of pmcTyr1. The decreased tyrosinase activity in the F10DD cells cannot therefore be explained by reduced transcription of the tyrosinase gene and is likely to result from some post-translational change. A defect in the post-translational activation of tyrosinase by cAMP is unlikely since 8-bromo cAMP (10^{-5}-10^{-3}M) stimulated tyrosinase activity in the F10DD cells and the percentage increases were identical to those in the F10 cells. The basal levels of cAMP in the F10DD cells were also similar to those in the F10 cells (25 ± 8 and 21 ± 2 pmol/mg cell protein/30 min respectively) and in both sublines showed large increases in response to α-MSH (10^{-8}M) and forskolin (10^{-5}M).

Thus while a reduction in tyrosinase activity explains the low level of melanin production in the F10DD cells the mechanisms responsible for this reduced enzyme activity are not altogether clear. Since the amounts of tyrosinase and its activation by cAMP appear to be normal it is possible that within the F10DD cells inhibitory mechanisms operate to suppress tyrosinase activity.

THE EFFECT OF COMMERCIAL SUN-BLOCKS IN PREVENTING UV DAMAGE TO LANGERHANS CELL FUNCTION IN VIVO.

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4 commercially available, factor 15 sunblocks - 2reflectant (A and B) and 2 absorbant (C and D) - were tested for their efficacy in preventing UV induced damage to Langerhans cells (LC) function. 7 healthy volunteers were used. The volar aspect of the left arm was initially irradiated with a bank of 3 Wolff Bellarium UV tubes, which emit approximately the same ratio of UVA and UVB as solar radiation, to assess the MED. SUbsequently, the right forearm eas fitted with a sheild with 6 cut out squares - one was blanked off, one untreated and sunblocks A,B,C and D were used under the other 4 squares. The arm was then irradiated for 3 MEDs. The arm was washed and the epidermis removed from each square using a 1cm suction blister cup. The epidermis was cold trypsinised and the single cell suspension of epidermal cells used as stimulator cells in a standard allogeneic response assay against peripheral blood mononuclear cells from 3 different volunteers.

Initial studies showed that at 3 MEDs, the LC activity was reduced by about 50% from that of unirradiated (blanked off square) LC. Sunblocks A,C and D showed no significant reduction in LC function after irradiation compared to the unirradiated LC. Sunblock B however showed a marked and statistically significant reduction of LC function to 60% of the unirradiated LC.

This study shows that sunblocks A,C and D do afford protection of LC function to UV irradiation at moderate doses. Sunblock B, however, failed to provide significant protection. Such sunblocks may cause major problems in preventing the acute sunburn effects of UV exposure, but allowing immunological damage to occur.

BASAL CELL CARCINOMA DERIVED FACTOR(S) INDUCE FIBROBLASTS TO MODIFY THE EXTRACELLULAR MATRIX.

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Basal cell carcinomas (BCC's) typically produce locally destructive invasive lesions. The mechanisms involved in this invasive process are unclear, but undoubtedly involve the release of collagenases, and as has been demonstrated, the production of factor(s) which stimulate fibroblast collagenase synthesis. Here we have examined the ability of BCC's to modify the extracellular matrix by investigating the expression of tenascin around tumour islands, the interaction of freshly excised tumour with fibroblast-contracted collagen lattices, and the effect of tumour tissue-conditioned medium on fibroblast glycosaminoglycan (GAG) synthesis.

Frozen sections of freshly excised BCC's were stained with polyclonal antitenascin using the immunoperoxidase method, while in vitro interactions were examined by incorporating BCC fragments within a fibroblast-contracted collagen lattice. BCC conditioned medium was used in fibroblast cultures radiolabelled with ^{3}H-glucosamine and $^{35}SO_4$ for GAG estimation. GAGs were isolated by ion-exchange chromatography and characterised by their susceptibility to degradation by specific enzymes.

Tumour tissue stained with antitenascin showed intense staining around tumour islands but not within the tumour nests or surrounding dermis. Inclusion of BCC fragments within collagen lattices resulted in degradation of the lattice following 5 days incubation. BCC-conditioned medium stimulated a 2-fold increase in the incorporation of ^{3}H-glucosamine into both sulphated GAGs and hyaluronate, while incorporation of $^{35}SO_4$ into sulphated GAGs remained essentially unaltered, with the net effect of an increased synthesis of low-sulphated GAGs. Serum-free tumour conditioned medium was found to promote fibroblast contraction of collagen lattices.

The observed changes in extracellular matrix induced by BCCs may facilitate their growth and invasion. Expression of tenascin has been associated with epithelial hyperproliferation while matrices rich in hyaluronate and low sulphated GAGs promote cellular migration.

EFFECTS OF A BRADYKININ ANTAGONIST, NPC567 ON HUMAN SKIN

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Bradykinin (BK) is known to be a mediator of oedema in the skin, and may also mediate pain and inflammation. NPC567 is a BK receptor blocker and if it antagonised the actions of BK in the skin it might be a useful anti-inflammatory agent.

The ability of NPC567 to antagonise BK was assessed in 21 normal volunteers. Either NPC567(1-500micromolar,µm) or phosphate buffered saline(PBS) was applied single-blind to 6 standardised 0.5cm. diameter abrasions . After 5 minutes 5ul BK(3-300µm) were applied. Pain was scored on a visual analogue scale from 0-10, and areas of weal and flare were measured. Pain from BK was reduced by higher concentrations of NPC567.

RATIO OF CONC. BK TO NPC567	ABRASION NUMBER	SCORE OF BK-INDUCED PAIN WITHOUT NPC567	WITH NPC567	
≤5	24	3.30 ± 1.5	3.24±1.5	0.5>P>0.1
5	8	3.30 ± 1.9	2.11±1.5	0.1>P>0.05
10	12	3.98 ± 2.3	3.25±2.0	0.05>P>0.02
20	6	6.33 ± 1.4	2.90±1.4	0.02>P>0.01
≥40	5	5.30 ± 2.7	3.44±2.7	0.1>P>0.05

Flare from BK was minimal; wealing occured at concentrations of BK from 3-300µm but never exceeded abrasion size by more than 3mm. The effects of NPC567 on BK-induced weal and flare were totally obscured by the flare and weal caused by the antagonist. This unexpected effect was markedly suppressed by oral terfenadine 60mg.

NPC567 (250um), FLARE AREA(cm^2) n=6

Control	Terfenadine	
14.22±3.31	1.53±0.80	P 0.001

Whilst we have shown that NPC567 reduces BK-induced pain, its ability to release histamine would limit any clinical applications.

DISTRIBUTION AND SIGNIFICANCE OF TONOFILAMENT CLUMPING IN THE DOWLING-MEARA FORM OF EPIDERMOLYSIS BULLOSA SIMPLEX (DM-EBS)

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The relationship between the mode of blister formation in the mechano-bullous disease DM-EBS, and the nature of the characteristic cytoskeletal abnormality, tonofilament (TF) clumping, was investigated. A total of 13 DM-EBS patients was studied, 2 of whom were neonates who died of extensive disease. Lesional, peri-lesional and non-lesional skin samples were examined by light microscopy (semithin sections) and by electron microscopy. All lesional samples (13/13) showed TF clumps extending from the basal to the mid-spinous epidermal layer. The level of blistering was invariably very low and just above the dermo-epidermal junction. Blisters formed mainly through cytolysis; however, acantholysis was also noted in 3 patients, (2 with severe disease). TF clumping was seen in 7/10 peri-lesional and 4/7 non-lesional samples. TF clumps always occurred in interfollicular epidermis but were also noted in hair follicles, sebaceous glands and sweat ducts. Cultured keratinocytes (feeder layer method) from 2 patients also demonstrated characteristic clumps. The number, size and morphology of the clumps did not appear to correlate with clinical expression. In conclusion, the presence of TF clumps in non-lesional skin and cultured cells suggests that clumping is a primary abnormality rather than a consequence of blistering. The intrinsic cytoskeletal defect is likely to result in impaired resistance of basal epidermal cells to external shearing forces, leading to cytolysis or more rarely, acantholysis.

CALCIUM DEPENDENT LOCALISATION OF CALGRANULINS IN KERATINOCYTES IN INFLAMMATORY DERMATOSES.

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The calgranulins are intracellular calcium binding proteins which inhibit leucocyte motility and regulate protein kinases. We have previously shown that these molecules are induced in keratinocytes in inflammatory dermatoses. They can also be induced in tissue culture of epidermis within 24 hours. In this study we have investigated the subcellular distribution of these molecules in keratinocytes by immunlocalisation methods and confocal laser scanning microscopy. The calgranulins in keratinocytes in dermatoses and in tissue culture were found in a punctate morphology at the cell periphery. A similar distribution was found with the plaque proteins of desmosomes (DPI + II). During the in vitro induction of the calgranulins the cytoskeleton underwent a redistribution to form a perinuclear halo seen by both electron microscopy and by confocal microscopy using anti-keratin antibodies. The calgranulin association with the plaque-like structure resisted extraction by non-ionic detergent in the presence of calcium but not in its absence.

We conclude that in keratinocytes calgranulins are bound to a subcellular plaque component of the detergent resistant cytoskeleton in a calcium dependent manner and that they may contribute to the cytoskeletal changes which accompany inflammatory dermatoses.

LONG TERM STUDIES IN BULLOUS PEMPHIGOID: CHARACTERIZING THE TARGET ANTIGEN(S).

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Twenty bullous pemphigoid patients were studied prospectively to characterize the number of target antigens involved. Changes in the target antigen(s) during the course of the disease were sought in relation to body site and localization within the basement membrane zone.

Sequential sera were collected over a period of approximately 2 years. They were investigated by standard immunofluorescence technique using thigh and breast skin and vaginal and oral mucosa. Split (1M NaCl) and intact tissue was used from all sites.

All patients had linear staining of the basement membrane zone in all sites on indirect immunofluorescence. In both split skin and mucosa all sera consistently detected an antigen located on the epidermal side regardless of the stage of the disease.

The concentration of circulating antibodies detected is similar in intact skin and intact mucous membranes. There was a tendency for skin to be more sensitive than mucosa. In addition split tissue was even more sensitive (17/19 patients) with detection of circulating antibodies at a two to three times greater dilution. There were striking exceptions; in 8/19 patients split vagina and occasionally split oral mucosa (in the same patients) were much less sensitive than all other tissues. This was a constant feature for an individual patient which did not alter with the stage of the disease. These results strongly suggests heterogeneity of antigens between tissues.

There was no correlation between antibody titre and severity of the disease in the group as a whole. However, within an individual patient there was a rough correlation between antibody titre and disease activity (14/17). There were notable exceptions, with the antibody titre being totally independant of disease activity.

We conclude that split skin is the most sensitive tissue to detect circulating antibodies in BP. Within an individual the fluctuation of the antibody concentration roughly correlates with the disease activity, but the absolute antibody level and comparisons between patients is misleading. The target antigens appear to be heterogenous and immunoblotting studies are in progress to confirm this. Preliminary immunoblotting results also suggest there may be a change in the antigen with time.

THE EXPRESSION OF RETINOIC ACID RECEPTORS IN HUMAN SEBACEOUS GLANDS

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Although all-trans retinoic acid is the most biologically active of the retinoic acid isomers, 13-cis retinoic acid is more effective in the treatment of acne. The mechanism of this effect is unknown, although we have suggested (Latham, Redfern & Thody, Br.J.Derm., 122:259,1990) that differences in the rates of metabolism of different isomers could explain the different pharmacological properties of 13-cis and all-trans retinoic acid. It is possible that some of the biological effects of retinoids on sebaceous glands are mediated by retinoic acid receptors and we have therefore compared the expression of RARs in sebaceous glands, skin and skin cell types. Sebaceous glands were dissected from male chest skin and the RNA extracted by homogenization in guanidinium thiocyanate and purification through CsCl. Northern blots of RNA were probed with RAR-α, RAR-β and RAR-γ probes. The expression of all three RARs was detectable in sebaceous gland RNA. Similarly, the expression of all three RARs was detectable in adult human skin, but in whole skin and sebaceous glands RAR-β is apparantly expressed at a lower level. Conversely, the expression of RAR-β was undetectable in cultured keratinocytes and some dermal fibroblast cultures.

THE CELL KINETICS OF SEBORRHOEIC WARTS AND THE SKIN ON WHICH THEY ARISE.

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Seborrhoeic warts (basal cell papillomas) are an enigma. They may represent localised benign epithelial tumours but can develop rapidly and in large numbers. Numerous questions are provoked by these lesions but in this study we have confined ourselves to three: (1) Are solitary lesions similar to lesions which appear in great numbers? (2) Is there a generalized epidermal abnormality in individuals who develop multiple lesions? (3) Are irritated basal cell papillomas different to non-irritated lesions from the cell kinetic viewpoint?

Fourteen patients (8 female, 6 male, mean age 59 years) with seborrhoeic warts (mean number 29 lesions) had a lesion excised and biopsies taken from perilesional skin and uninvolved buttock skin. Half of each specimen was processed for routine microscopy, and half was incubated in the presence of tritiated thymidine and prepared as an autoradiograph. The proliferative activity of the tissue was estimated by calculating the labelling index (LI), the number of labelled cells expressed as a percentage of the number of basal cells.

The basal cell papillomas showed a similar range of histological patterns whether excised from patients with large numbers of lesions or with very few. There was no significant difference between the mean epidermal thickness of perilesional skin (59.7± 8.0 µm) and uninvolved skin (54.1±2.4 µm), and no gross morphological differences. The LI of the lesions was similar to normal skin although higher in irritated lesions. The perilesional skin appeared to have a higher LI than normal or lesional skin but analysis of variance revealed no significant difference between sites in patients with either few or multiple lesions.

These findings suggest that proliferative activity is not increased in basal cell papillomas. There was no significant difference in the kinetics of lesional or perilesional skin in any patients, including those with large number of lesions.

EXPRESSION OF 65 KILODALTON HEAT SHOCK PROTEIN IMMUNOREACTIVITY IN NORMAL AND PATHOLOGICAL SKIN.

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Heat shock proteins (hsp) are highly conserved between species, ranging from mammals to plants and microorganisms, and they are produced by cells in response to heat and certain noxious stimuli. The monoclonal antibody ML-30 (Ivanyi,J. et al, Clin.Exp.Immunol, 1983; 52: 528-536) identifies 65 kilodalton hsp (hsp 65), which may be recognised by the cellular immune system, in particular by T lymphocytes bearing gamma-delta antigen receptors (Hill,JS. et al, J.Exp.Med., 1990; **171**: 831-841). In certain microorganisms, hsp 65 has been characterised as a major antigen. We therefore investigated the expression of hsp 65 immunoreactivity in paraffin sections of normal and pathological skin by using ML-30 antibody (1 in 250 dilution) and an indirect immunoperoxidase technique.

Consecutive staining of tissue sections, using a different biopsy sample for each section, gave consistent results for the diseases studied. Normal skin demonstrated weak cytoplasmic staining of dermal and epidermal cells exceptcertain dermal mononuclear cells, which remained unstained. In lichen planus and bullous pemphigoid there was cytoplasmic labelling throughout the epidermis. In contrast, in psoriasis epidermal labelling was weak and seen mostly in the cytoplasm of suprabasal spinous cells. Intense cytoplasmic labelling was seen in the acanthotic epidermis of viral warts, whereas basal cell carcinoma cells were devoid of staining.

These findings suggest that hsp 65 has a specific distribution in normal skin, with variable expression in different inflammatory and proliferative skin diseases. Knowledge of differences in hsp 65 expression may provide further insight into the pathogenic mechanisms concerned.

AN IMMUNOHISTOCHEMICAL ANALYSIS OF THE CHANGES IN THE IMMUNE INFILTRATE AND KERATIN EXPRESSION IN PSORIASIS TREATED WITH CALCIPOTRIOL COMPARED WITH BETAMETHASONE OINTMENT.

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The vitamin D3 analogue, Calcipotriol is effective in treating chronic plaque psoriasis though its mechanism of action is unknown. We compared the effect of treatment with Calcipotriol and betamethasone ointment on the immune infiltrate and keratin expression as psoriatic plaques improved.

Psoriatic patients were treated in a randomised, double blind, parallel group study with a 50mcg/g Calcipotriol ointment (n=8) or 0.1% betamethasone valerate ointment (n=7) for 6 weeks. Psoriasis area activity scores were assessed at 0,2 and 6 weeks. At each visit 4mm punch biopsies were taken from the same plaque for immunohistochemical analysis of the immune infiltrate and keratin expression by an indirect immunoperoxidase method using a battery of monoclonal antibodies.

Similar clinical improvement was seen in both groups. Immunohistochemical analysis showed an equal reduction in epidermal acanthosis, parakeratosis and Langerhans' cells and a progressive reduction in the dermal perivascular infiltrate. Initially the infiltrate showed a predominance of CD4+ cells, but as the plaques improved there was a relative increase in the proportion of CD8+ cells. Abnormal keratin expression, including keratins 6 and 16, markers of hyperproliferation in psoriasis, persisted unchanged in both groups despite improvement in the immune infiltrate.

Our results show no discernable differences in the mechanism of action of Calcipotriol and betamethasone ointment. With both the immune infiltrate shows significant change whilst persistent abnormal keratin expression may be a reflection of the incomplete resolution of the clinical lesions.

USE OF HUMAN SKIN IN IN VITRO TESTING OF THE EFFICACY OF SUNBLOCKS IN PROTECTING LANGERHANS CELLS FROM UV INDUCED DAMAGE.

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In this study surgical skin samples were used to test the efficacy of sunblocks in preventing immunological damage to Langerhans cells (LC) function in vitro. Human skin removed at cosmetic surgical operations was collected immediately and washed in sterile PBS. The skin with underlying subcutaneous tissue was cut into 1.5cm squares and placed in sterile humidity chambers. Some squares were left untreated and others were treated with 4 commercially available, factor 15 sunblocks, 2 absorbant (A and B) and 2 reflectant (C and D). The squares were then irradiated for 0 to 200 minutes using a bank of 3 Wolff Bellarium tubes, which emit a similar ratio of UVA to UVB as solar radiation. In vivo testing showed that 1 MED equalled 15 or 20 mins for skin type 1 or 2. After irradiation, the skin was decontaminated and the epidermis cut away in strips cold trypsinised and a single cell suspension produced. These epidermal suspensions were used as stimulator cells in standard allogeneic response assays with peripheral blood mononuclear cells from 4 volunteers.

Results showed a gradual reduction of LC function with UV irradiation to about 50% reduction at 200 mins. Absorbant sunblocks A and B protected the skin from UV damage. Reflectant blocks C and D, however, failed to protect the LC from UV damage with a statistical reduction of function down to 50% of unirradiated LC.

This method of testing UV damage to LC function approximates the conditions in vivo. Testing of sunblocks with this method will identify those that fail to protect the cutaneous immune function.

A METHOD FOR DIRECT MEASUREMENT OF PHOSPHOINOSITIDE SPECIFIC PHOSPHOLIPASE C (PLC) ACTIVITY IN MONONUCLEAR LEUCOCYTES FROM NORMAL AND ATOPIC SUBJECTS.

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Intracellular secondary messenger system abnormalities, particularly elevated cAMP phosphodiesterase(PDE) activity, are present in mononuclear leucocytes(MNL) of patients with atopic dermatitis(AD). Previous studies of the inositol pathway used indirect methods to assay PLC. We have modified Bergers et al's method (Phosphotidyl inositol 4,5-bisphosphate phospholipase C activity in particulate preparations from rat brain. Lipids 24 13-16 1989) to measure directly membrane and cytosol PLC activity.

MNL were separated by Ficoll-hypaque centrifugation of venous blood from AD patients and normal controls. Membrane and cytosol fractions were formed by ultracentrifugation of sonicated MNL homogenates. PLC assay employed ^3H-phosphotidyl inositol 4,5-bisphosphate(PIP_2) as substrate in a micelle suspension formed in the presence of 0.4mg/ml cetrimide. The optimal pH was 6.8 in a buffer containing 1mM $CaCl_2$, 25mM LiCl, and 1mM $MgCl_2$.

Hydrolysis of PIP_2 occurred over a range of substrate concentrations from 0.5-2.5nM in both membrane and cytosol fractions. Initial results comparing normal and AD MNL showed a significant increase of membrane bound PLC activity in MNL from AD sufferers. (AD($n=14$) 42.8$\pm$ 7.56; normal($n=13$) 32.3$\pm$8.82 $p< 0.0025$; pMol PIP_2 hydrolysed/min/10^6 cells at a substrate concentration of 2nM$\pm$ S.D.) but with a corresponding decrease in cytosol activity.

Elevation of membrane bound PLC activity in AD MNL could lead to activation of protein kinase C by diacylglycerol and lead to an increase in PDE activity as has been previously reported in AD.

AN ULTRASTRUCTURAL STUDY OF THE DERMAL-EPIDERMAL JUNCTION IN NORMAL AND ALOPECIA AREATA HAIR FOLLICLES.

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The dermal papilla (DP) is thought to regulate many parameters of normal hair growth; in alopecia areata (AA) these mechanisms are altered. The dermal-epidermal junction (DEJ) in skin is well known to be characterised by the presence of hemidesmosomes (HD), but the situation in the hair follicle is unclear. In this study we have examined the dermal papilla-epithelial junction (DPEJ) of the human hair follicle to determine how it compares with that of the skin. A basic understanding of the DPEJ is necessary before diseases of the hair follicle eg. AA can be better understood.

31 follicles were dissected from 16 biopsies taken from active and inactive regions of AA Scalp; 7 anagen follicles from 5 biopsies of the occipital/parietal area of healthy scalps were used as controls. All follicles were routinely processed for transmission electron microscopy. Vertical ultrathin sections from the middle of the follicles were used in this study.

The DP and epithelial cells are separated by a tri-lamina basement membrane. Junctions in the DPEJ did not resemble those of the DEJ but were similar to the modified HD seen between melanocytes and connective tissue matrix in skin. This difference probably reflects the relatively permanent state of the epidermis compared to the fluctuations of the hair cycle. In patients with AA the number and condition of junctional features was dependent on the activity of the clinical disease. These results suggest that the condition of the attachment points is important to the regulation of hair growth.

INHIBITION OF SKIN FIBROBLAST METABOLISM BY MINOXIDIL

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The demonstration that minoxidil (MX) inhibits the enzyme lysyl hydroxylase, which is required for collagen cross-linking, has suggested the topical use of MX for keloids and other local fibroses (Pinnell & Murad, Dermatologica 1987; 175: suppl. 2: 12-18). We have studied some other effects of the drug on skin fibroblasts in vitro, using cells grown from patients with morphoea (MF), lichen sclerosus et atrophicus (LSAF) and from normal subjects (HSF).

Proliferation of all cell lines over 3 days was inhibited in proportion to concentration of MX, down to 20% or less of untreated controls at 1mM, where cell viability was only slightly reduced (84 ± 2% versus 88 ± 2% (s.e.m.) in controls; $p>0.2$). At 5mM there was often a net loss of cells, and only 72% of those remaining were viable. In contrast MX at 0.1 - 1mM stimulated the proliferation of foreskin keratinocytes by up to 130%. Contraction of collagen lattices containing MF, LSAF or HSF was inhibited by 25, 22 and 26% with 1mM MX after 5 days and by 50, 94 and 79% with 5mM MX. Glycosaminoglycans secretion by HSF cells (as uronic acid per 10^7 cells) showed a concentration-dependent reduction to 25 ± 6% of that of untreated cultures at 1mM and was zero at 5mM. These findings show that MX has a range of inhibitory effects on fibroblasts in vitro, which contrast with its stimulation of skin epithelial cells, and support suggestions that it might be a useful treatment for fibroses.

EFFECT OF GONADOTROPHIN RELEASING HORMONE ANALOGUE (ZOLADEX) ON SEBUM EXCRETION RATES AND HAIR SIZE IN HIRSUTE WOMEN.

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Hirsuties has been attributed to excessive androgen metabolism within skin although most women with hirsuties have normal or only slightly elevated plasma levels of testosterone. Gonadotrophin releasing hormone (GnRH) analogues abolish ovarian sex steroid production and this study examines their effect, with oestrogen replacement, on sebaceous gland activity and hair growth in a group of normal cycling mildly hirsute women.

Sixteen patients with idiopathic hirsuties, (aged 28 years: range 19-4[illegible]) with normal menstrual cycles (cycle ranged 21-42 days), adrenal function, luteinising hormone and a body mass index between 19 and 25 were studied. Patients received six depot implants of GnRH analogue (Zoladex, ICI) 3.6 mg subcutaneously at 4 weekly intervals initiated in early follicular phase as well as a single 25 mg oestradiol subcutaneous implant at the time of the first Zoladex implant. Hair measurements, by a global scoring system (Ferriman and Galwey; F&G) and microscopy (hair diameter, growth rate and presence of medulla), sebum excretion rates (SER in μg.min^{-1}.cm^{-2}), and measurements of circulating testosterone (nmol/l), sex hormone binding globulin (nmol/l)(SHBG), and free androgen index (FTI) were made at 8 weekly intervals.

The treatment was well tolerated. Seven patients had amenorrhoea and the remaining 9 had reduced menstrual bleeding. Only one patient complained of mild flushing. Patients did not notice any significant improvement in their hirsuties over the active study period. There was a 10-15% fall in SER compared with baseline during the treatment period (week 8 to 24) with a rebound 8 weeks after the last treatment but these changes did not reach statistical significance (table). There was no consistent change in hair diameter or hair growth rate nor in the numbers of hair in which the medulla was intact, partial present or absent. There was a significant change in F&G hirsuties score during the treatment period (*$p<0.05$, wilcoxon statistic).

This study has demonstrated that although the GnRH analogue caused suppression of testosterone and FTI this effect was not sufficient to affect hair growth rate or hair diameter. The effect on sebum excretion rate was no more than a trend towards suppression but no patient showed an increase.

	SER	**Testosterone**	**FTI**	**SHBG**	**F&G**
Baseline	1.13	0.65	63	42	12.4
8 wks	0.99	0.39*	41*	44	10.3*
16 wks	0.94	0.33*	35*	45	10.3*
24 wks	1.00	0.34*	32*	45	10.0*
8 wks post	1.30	0.51	43	40	11.0
16 wks post	-	-	-	-	13.5

THE MANNER OF PLAQUE CLEARANCE DURING ANTHRALIN THERAPY.

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The aim of this study was to find out whether psoriasis clears by reduction in plaque thickness or plaque area and thereby to establish which to measure when monitoring treatment response. 9 inpatients with psoriasis (4 females and 5 males; age range 18-48, mean 30 years) receiving Ingram anthralin treatment were studied. Each patient had the lesional and perilesional skin of 3 plaques studied at fixed skin sites, to enable accurate measurements of involved skin before and at weekly intervals during anthralin therapy by the following methods: plaque thickness was measured by the difference between lesional and perilesional skin using ultrasound, Harpenden calipers and transepidermal evaporative water loss (Tewl-Servomed evaporimeter). The area of each plaque was measured in 3 ways: a tracing onto polythene, a tracing of plaque outline taken from a photograph of the untreated plaque and a tracing of a photograph of the plaque after application of anthralin in Lassar's paste. The areas of all tracings were measured using a computer digitising pad. 23 of the 27 plaques cleared and there was a gradual decrease in plaque thickness shown by all 3 techniques. Tewl decreased from a pre-treatment mean initial value of 22.4gm^{-2}hr^{-1} (SEM ±0.5) to 6.6(±1.5), 8.4(±1.5), 2.09(±0.4) and 2(±0.001) at 1,2,3 and 4 weeks respectively. Similarly ultrasound mean pre-treatment value was 0.9mm(±0.1 SEM) and decreased to 0.52(±0.15), 0.53(±0.11), 0.4(±0.1) and 0.14mm(±0.001) at weeks 1,2,3 and 4 respectively. Plaque area steadily decreased throughout the treatment period with a pre-treatment mean value for the polythene tracing method of 497mm^2 (±131 SEM) reducing to 87(±37), 139(±92), 57(±43) and 0mm^2 at weeks 1,2,3 and 4. There was close correlation between the polythene tracing method and the other 2 methods used to estimate area. The area derived from photographs of plaques treated with anthralin provided excellent colour contrast and would have application in image analysis quantification of psoriasis extent. The study shows that psoriasis clears by a concurrent decrease in both plaque thickness and area when treated with anthralin and therefore new quantitative measures of therapeutics in psoriasis should include both of these aspects.

EFFECT OF TOPICAL STEROID ON THE RECOVERY OF INTERLEUKIN-1 FROM NORMAL HUMAN SKIN.

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Interleukin-1 (IL-1) has been recovered from normal human skin where it is presumed to be biologically unavailable, possibly through intracellular storage. Once released IL-1 may play an important role in cutaneous inflammation. As topical steroids are effective in treating a variety of dermatoses, we wished to determine their effect of on the production of IL-1 in human skin.

Nine volunteers applied betamethasone valerate 0.1% ointment and vehicle alone, twice daily to symmetrical marked areas on both thighs, under double blind conditions. After 2 weeks (3 subjects) and 6 weeks (6 subjects), three 19mm internal diameter skin chambers were applied, to the steroid and vehicle treated sites and to an untreated site, following sellotape stripping of 2cm diameter areas. Phosphate buffered saline (1ml) was added to the skin chambers, removed after 30 min and analysed for the presence of IL-1 in an EL-4 NOB-1 bioassay (Gearing et al., J Immunol Methods 99:7, 1987). Preliminary experiments were performed to ensure that ointment contaminating skin chamber fluid did not interfere with the assay.

There was no difference in the levels of IL-1 activity in samples from steroid and vehicle treated sites at 6 weeks. However higher levels of activity were found in samples from untreated compared to steroid or vehicle treated sites, similar findings were obtained with samples recovered at 2 weeks.

	n	steroid[1]	vehicle[1]	untreated[1]
2 weeks	3	2.3±1.1	3.3±0.8	5.6±2.4
6 weeks	6	2.5±1.3	2.8±1.5	5.1±1.6[2]

[1] IL-1 equivalents (ngml^{-1}, means±s.e.m.), determined by reference to a standard dose-response curve generated with recombinant IL-1.

[2] p=0.02 on comparison with steroid value (Wilcox on Signed Rank Test).

In contrast to the reported effects of corticosteroids on IL-1 synthesis in vitro, prolonged application of topical steroids to human skin has no greater influence on IL-1 activity than vehicle. The sustained anti-inflammatory effectiveness of topical steroids can therefore not be ascribed to suppression of IL-1 production or release. The therapeutic effectiveness of ointments may however be due in part to diminished IL-1 release.

FOETAL DEVELOPMENT OF EPIDERMAL LANGERHANS CELLS AND T CELLS.

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Skin from different sites of the body and different foetal ages were examined for the presence of Langerhans cells and T cells. Foetal tissue was obtained from 22 theraputic termanations of pregnancy, 3 spontaneous abortions at 20, 21 and 24 weeks and one full term stillbirth. The foetal age in the theraputic terminations was measured by ultrasonic assassment of foetal length in utero and ranged from 8 to 18 weeks. Tissue was taken from various sites and snap frozen. Cryostat sections were stained with monoclonal antibodies against DR, CD1, $\gamma\delta$ -TCR and CD3 using an APAAP technique.

DR+ cells were first observed at 9 weeks. These cells were rounded and irregularly spaced. At 10 weeks DR and CD1+ cells were seen in equal numbers and numerous CD1+ cells were observed in the dermis. By 14 weeks the cells were more dendritic and regularly spaced and were present at a density of 4/mm epidermis. By 18 weeks the density of CD1+ cells was 9/mm epidermis which remained fairly constant to term. The numbers of CD1 and DR+ cells were equal at 10, 13 and 14 weeks but by 15 weeks the number of DR+ cells decreased and this was maximal at 17 weeks where DR+ cells were 1.5/mm epidermis compared to CD1 at 5/mm epidermis. By 20 weeks, the DR and CD1+ cells were equal. Due to the low numbers of Langerhans cells present in early foetal stages it was impossible to assess regional variation of density. In older foetuses, higher density of Langerhans cells were seen in skin from the back and foot.

$\gamma\delta$ - Tcells were only infrequently seen in the dermis and were not observed in the epidermis. CD3+ cells were seen but these cells were sparse and irregularly distributed.

SULPHATED GLYCOSAMINOGLYCANS IN THE EXTRACELLULAR MATRIX AND MEDIUM OF CULTURED HUMAN HAIR PAPILLA AND CONNECTIVE TISSUE SHEATH CELLS

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Histochemical and immunohistochemical studies have shown the dermal papilla (DP) and connective tissue sheath (CTS) of anagen follicles to contain an extracellular matrix (ECM) rich in sulphated glycosaminoglycans (GAG). Staining for GAG diminishes during catagen and is minimal or absent in telogen. To further study the role of GAG in hair growth we have compared the incorporation of radiolabelled sulphate into GAG synthesised by cultured human DP cells, CTS cells and interfollicular dermal fibroblasts (DF).

Twelve DP, 8 CTS and 14 DF cell lines were studied. Subcultured cells from primary explants were grown to confluence in 24-well plates in E199+10% foetal bovine serum. Quadruplicate wells were labelled with S^{35}-sulphate (3MBq/ml) for 72 hours. Cell counts were performed on quadruplicate unlabelled wells. Medium was removed and the cells lysed with 0.1% Triton X-100 for protein assay. Medium and subcellular ECM were digested with pronase to release GAG from proteoglycans. Analysis of total incorporation into GAG was performed by spotting pronase-digested material onto cellulose acetate and processing to liquid scintillation counting.

Incorporation of S^{35}-sulphate into ECM GAG was significantly higher ($p<.01$) in DP cells (14.1 ± 2.0 dpm/µg cell protein/10^{3}cells, $\bar{x}$ ± S.E.) and CTS cells (16.0 ± 2.4) than DF cells (4.9 ± 1.1). Incorporation into medium GAG was similar in the three cell types (DP = 41.2 ± 6.9, CTS = 52.8 ± 7.5, DF = 36.9 ± 4.8).

Thus, whilst the overall incorporation of radiolabel into sulphated GAG in the medium was similar in the three cell types, cells derived from hair follicle mesenchyme assembled a much higher proportion than non-follicular fibroblasts into an extracellular matrix. The reason for this difference may have an important bearing on the regulation of ECM in hair follicle mesenchyme.

TYROSINASE EXPRESSION IN HUMAN HAIR FOLLICULAR MELANOCYTES AND ITS RELATIONSHIP TO THE SYNTHESIS OF EUMELANIN AND PHAEOMELANIN

S.A. Burchill, S. Ito[1] & A.J. Thody, Dermatology Dept., University of Newcastle upon Tyne and [1]Chemistry Dept., Fujita-Gakuen Health University, Japan.

Tyrosinase is a rate limiting enzyme in the melanin pathway. In this study we have examined its expression in eumelanin and phaeomelanin producing human hair follicular melanocytes.

Samples of hair were obtained from 13 red haired and 10 brown or black haired Caucasian subjects. The concentrations of eumelanin and phaeomelanin determined by the method of Ito & Fujita (Anal.Biochem. 114: 527-536, 1986) confirmed that dark hair contained more eumelanin and red hair greater amounts of phaeomelanin. Tyrosinase synthesis was measured in anagen hair bulbs, obtained from the scalp by plucking, by immunoprecipitation of [^{35}S]methionine labelled proteins (Burchill et al, J. Endocr. 116: 17-23, 1988) and when expressed as dpm of [^{35}S] labelled tyrosinase/dpm of labelled total protein x100, ranged from 2.0-4.0 in the dark haired subjects and from 3.0-12.0 in the red haired subjects. Tyrosinase activity was also higher in the hair bulbs of the red haired subjects than those of dark haired subjects (4.4 ± 0.2 v. 0.88 ± 0.06 pmol tyrosine converted/h). Tyrosinase synthesis correlated well with the levels of phaeomelanin particularly in the red haired subjects ($r=0.8066$) but in the dark haired subjects showed a better correlation with eumelanin ($r=0.6984$). The presence of α-MSH (10^{-4}M) in the incubation medium had no effect on tyrosinase synthesis. Cyclic AMP (10^{-2}M) on the other hand, increased tyrosinase synthesis but only in hair follicles from dark haired subjects. These findings indicate that tyrosinase synthesis may represent a control point in the expression of tyrosinase in hair follicular melanocyte and that differences at this level may be related to the type of melanin produced by these melanocytes.

CHARACTERISATION OF GLYCOSAMINOGLYCANS IN ISOLATED HAIR FOLLICLES L.P. Watson, G.E. Westgate, M.A. Blount, W.T. Gibson. Personal Products Section, Unilever Research, Colworth House, Sharnbrook, Bedford, U.K.

In previous studies we have investigated the distribution of proteoglycans (PG) during the hair growth cycle in rat and human skin using immunostaining techniques. We noted a loss of chondroitin PGs from the connective tissue sheath and dermal papilla during catagen and we postulated that these PGs may have an important role in the growth stage of the cycle. We aim to use in vitro techniques to further investigate the role of PG in hair growth and present here a characterisation of the PG content of isolated rat follicles using biochemical methods.

Follicles were isolated from rat pelage skin and cultured for 48hr. For metabolic studies, 50-100 follicles were labelled with either 35S sulphate or 3H glucosamine for the final 24hr of culture then washed and pronase digested to release the GAG chains. Free label was removed using DEAE sephacel. The glycosaminoglycans (GAG) were characterised using specific enzyme and acid treatments followed by analysis on Mono Q FPLC.

Results from sulphate labelling show that in culture, rat hair follicles produce chondroitin, heparan and dermatan sulphates and the 3H glucosamine labelling shows that the follicles are able to synthesise hyaluronan. In initial studies on human follicles, we have demonstrated the presence of sulphated GAGs using both biochemical and immunostaining techniques.

These models will enable us to further our studies into the importance of GAGs for hair growth.

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Editor

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Correspondence

Increased frequency of HLA-DR2 in patients with Darier's disease

SIR, We previously reported a patient with Darier's disease (DD) and primary Sjögren's syndrome (SS).[1] Darier's disease is a rare genodermatosis with autosomal dominant inheritance and incomplete penetrance and a variable age of onset.[2] In addition, the oral mucosa and eyes may be affected.[2] Primary SS is characterized by an autoimmune-mediated progressive destruction of exocrine glands with a keratoconjunctivitis sicca and xerostomia.[3] In patients with primary SS there is a significant increase in the frequency of HLA-DR2 and -DR3.[4] This study was carried out to determine whether there is a relationship between DD and SS.

Fourteen unrelated Danish patients with DD were typed for all the major HLA-subtypes (A, B, C, DR, DQ and DP) and 11 of them were included in a clinical study recording their symptoms and the results of eye examinations (Schirmer test, break-up time and Rose Bengal score). Serological investigations were carried out for antinuclear antibodies, immunoglobulins and phase reactants.

In eight patients HLA-DR2 was found, showing an increased frequency of 57·1% compared to 28·3% in a population of 704 unrelated Danes[5] ($P = 0{\cdot}024$, Fisher's exact test). No other HLA associations were found. Six patients (four on low-dose etretinate therapy) had eye or mouth symptoms and/or abnormal ocular tests, and/or increased circulating antinuclear antibodies. A biopsy of the lower lip of one patient, who had a dry mouth and two out of three abnormal eye tests, was found to be normal. None of the patients fulfilled the criteria for SS.[3] The HLA-DR2 antigen has been found in association with autoimmune diseases[6] and immunological abnormalities have been reported in patients with Darier's disease.[7,8] We suggest that DD may be associated with HLA-DR2, but because of the small number of patients included in the study and the borderline *P* value, further studies are indicated.

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Sensitization as a result of a religious ritual

Sir, Perfumes are well recognized as contact allergens,[1] but to our knowledge sensitization as a result of a religious ritual has not been described. We report a case of chronic hand eczema in a Bangladeshi Muslim, previously elusive to diagnosis, which on investigation was shown to be due to ceremonial perfume applied as a purification ritual before Friday prayers.

A 49-year-old man presented with a long history of eczema affecting the pulps of the index, middle and ring fingers of his dominant right hand and the centre of the left palm. The condition had fluctuated in severity, but he had never been free of hand eczema for 25 years. The patient had a background history of asthma and hay fever and in 1981 was diagnosed as having atopic hand eczema. The patient was applying no topical agents and denied a history of relevant contact factors.

Patch-testing to the standard European battery and the perfume series produced erythema and vesiculation at 48 and 96 h to balsam of Peru, perfume-mix, cinnamyl alcohol and cinnamaldehyde, indicating an allergic contact dermatitis to perfume. Further close questioning revealed that as a devout Muslim, before the main prayers of the week on Fridays, he adhered to the advice of Mohammed: 'bathe, and if anyone has perfume it does him no harm to apply some of it',[2] pouring it into his left palm and applying it to his clothes with the fingertips of his right hand. Subsequent patch-testing to the ceremonial perfume was positive and avoidance of the practice resulted in clearance of his hand eczema after 6 weeks, for the first time in 25 years.

We report this case to illustrate the importance of being aware of the habits and customs of ethnic minorities.

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Varicella bullosa in an adult

Sir, We wish to report a case of a 33-year-old woman with varicella bullosa. Four days before admission to our hospital she developed papules and vesicles on her face. Two days after the onset the vesicles became generalized and giant bullae developed on the upper half of the body which varied in size from 2 to 6 cm. There were vesicles and pustules 2–3 mm in size with an erythematous base on the whole of the body and flaccid pustular lesions on the face (Fig. 1), neck, chest, upper back, right axilla and left forearm. She had no past history of varicella.

Investigations performed on her were as follows: WBC 8300/mm^3 with 32% atypical lymphocytes which after 3 days declined in number to 2%; the serum immunoglobulins were IgG 1430 mg/dl, IgA 239 mg/dl, IgM 239 mg/dl. Virus isolation was performed on the contents of the vesicles and the giant pustules using human embryonic lung fibroblast cultures. Positive cultures were obtained from the vesicles but not from the giant pustules. However, a few varicella zoster virus-specific-antigen-positive epidermal cells were observed in smears obtained from the contents of the giant pustular lesions (Fig. 2). Anti-VZV monoclonal antibody (Biosoft) and FITC-conjugated anti-mouse IgG antibody (Cappel) were used for the detection of VZV-specific antigen. Bacterial cultures confirmed the presence of *Staphylococcus aureus* in the giant pustules. This was classified as coagulase type V and phage group II 3A/3C/71. Exfoliative toxin production was also demonstrated by this strain on the skin of newborn mice. Our patient was treated with acyclovir and intravenous cefuzonam sodium followed by oral cefaclor with resolution of the lesions.

The histology of a biopsy of a giant pustular lesion on the left forearm showed a split in the granular layer

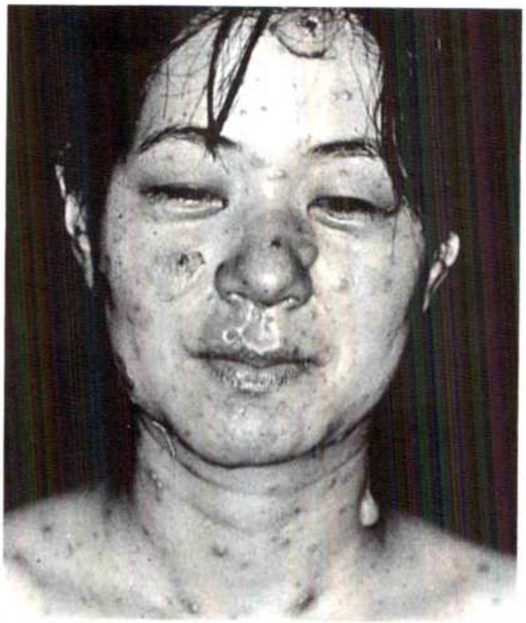

FIGURE 1. Giant pustules of varicella bullosa on the face.

of the epidermis with intracellular oedema and acantholytic cells below. These findings can be found in impetigo and in the staphylococcal scalded-skin syndrome.[1]

Varicella bullosa resembles ordinary varicella but is complicated by formation of giant bullae.[2] These are caused by superinfection with *S. aureus* producing an exfoliative toxin.[3,4] The condition is rare and usually affects infants and children,[2–4] and only two cases have so far been reported in adults.[5] In one of these cases, a 70-year-old man died from a bronchopneumonia. There was no evidence of extracutaneous varicella. The other patient was a 24-year-old woman who fully recovered from the condition. Although our patient temporarily had atypical lymphocytes in the peripheral blood, she did not develop any serious underlying

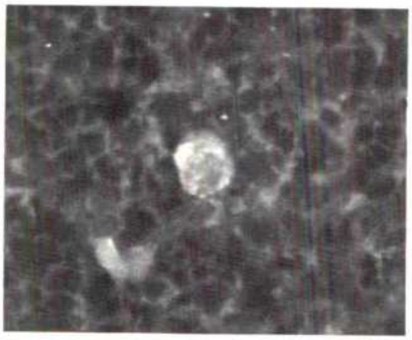

FIGURE 2. Varicella zoster virus-specific-antigen-positive epidermal cell in smears obtained from the contents of giant pustules.

disease after the widespread eruption had healed. Varicella bullosa in adults does not appear to be indicative of any underlying systemic disease which may occur in children.[5]

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British Journal of Dermatology (1990) **123**, 849.

News and Notices

6th Course in Paediatric Dermatology
9–10 May 1991, University of Liverpool

A 2-day course on Paediatric Dermatology will be held at the University of Liverpool on 9–10 May 1991. This is for recently appointed Consultants and Senior Registrars and is limited to 12 participants. Further details are available from: Dr J.L.Verbov, c/o Postgraduate Office, Faculty of Medicine, P.O. Box 147, Liverpool L69 3BX. Telephone 051-709-3114.

71st Annual Meeting of the British Association of Dermatologists
3–6 July 1991, London

The next annual meeting of the British Association of Dermatologists will be held in London on 3–6 July 1991 under the Presidency of Dr Harvey Baker. Dr Michael Klaber will be acting as Local Secretary. The scientific meeting will be held at Kensington Town Hall and on the morning of Saturday 6 July there will be a clinical meeting at the Royal London Hospital. There will be a short clinicopathological conference (CPC) on Thursday morning, 4 July after the AGM, and suitable case reports are invited.

Abstracts of papers and posters are invited for consideration by the Selection Committee and those accepted as papers will be allotted 10 minutes for presentation and time for discussion. Abstracts of papers, posters and CPC case reports should be submitted on the official forms which are available from: Mrs R.Barton, Conference Manager, British Association of Dermatologists, 3 St Andrew's Place, London NW1 4LB. The closing date for receipt of completed abstract forms is Friday 11 January 1991. Clinical summaries are invited for patients to be demonstrated at the clinical meeting on Saturday 6 July. The closing date for receipt of summaries is Friday 25 January 1991.

Index

SUBJECTS

Abrasive agents, effects on skin, 457
Acantholysis and the vagina (Correspondence), 691
Acanthosis nigricans-like epidermal naevus and Klippel–Trenaunay syndrome (Case report), 539
Acrodermatitis chronica atrophicans (Case report), Suppl. 37, 109
Acrodermatitis chronica atrophicans (Case report), 677
Alpha-MSH and cAMP and melanogenesis, 145
Alopecia areata, extracellular matrix and hair follicle mesenchyme, 717
Alopecia areata hair follicles, dermal–epidermal junction ultrastructure (Poster summary), 839
Alopecia areata and melanogenesis (Paper summary), Suppl. 37, 34
Alopecic (DEBR) rat, immunobiological studies, 557
Anetoderma and Borreliosis (Case report), 541
Anetoderma of Jadassohn—Pellizari (Case report), 686
Angiosarcoma (Case report), 537
Angiotropic B-cell lymphoma, 807
Anti-keratin autoantibodies, 735
Antinuclear antibodies and polymorphic light eruption (Paper summary), Suppl. 37, 14
Aquagenic pruritus and myelodysplastic syndrome (Correspondence), 414
Argyria, acquired localized, 403
Atopic dermatitis and activated T lymphocytes (Paper summary), Suppl. 37, 49
Atopic dermatitis, bacterial studies, 493
Atopic dermatitis and house dust mite patch tests (Paper summary), 675
Atopic dermatitis and topical nedocromil sodium (Poster summary), Suppl. 37, 60
Atopy and prostaglandin E defect (Correspondence), 126
Atypical histiocytosis and lymphomatoid papulosis, 515
Audit, dermatological (Paper summary), Suppl. 37, 20
Audit and psoriasis, 99

Balanitis of Lortat-Jacob and Civatte (Case report), 265
Bamboo hair, pathogenesis (Paper summary), Suppl. 37, 33
Basal cell carcinoma-derived factors and fibroblasts (Poster summary), 833
Bath PUVA for psoriasis (Paper summary), Suppl. 37, 42
Benign cephalic histiocytosis, 245
Benzoyl peroxide and carcinogenesis (Correspondence), 129
Beta-2-adrenoreceptors and cultured melanoma cells, 163
Betamethasone, effect on DNA content and Ks8.12 binding in psoriatic lesions, 291
Book Reviews:
  Acyclovir Therapy for Herpesvirus Infections. Edited by D.A.Baker, 132
  A History of St John's Hospital for Diseases of the Skin, 1963–1988. P.D.Samman, 418
  Andrew's Diseases of the Skin. Clinical Dermatology. 8th edn. H.L.Arnold Jr, R.B.Odom and W.D.James, 417
  Clinical Dermatopathology: a Text and Colour Atlas. A.Stevens, P.Wheater and J.Lowe, 278
  Essential Aspects of Atopic Dermatitis. G.Rajka, 278
  Flaps for the Central Face. S.J.Salasche and W.J.Grabski, 417
  Herpes Simplex Virus. A.Mindel, 695
  Irritant Contact Dermatitis, Edited by E.M.Jackson and R.Goldner, 132
  Management of Blistering Diseases. Edited by F.Wojnarowska and R.A.Briggaman, 418
  Mycobacterial Skin Diseases. Edited by M.Harahap, 280
  Occupational Skin Disease. 2nd edn. R.M.Adams, 279
  Onychomycoses: Local Antimycotic Treatment. Edited by S.Nolting and H.C.Kortin, 553
  Pharmacology of the Skin II. Edited by M.W.Greaves and S.Shuster, 278
  Recent Advances in Dermatology No. 8. Edited by R.H.Champion and R.J.Pye, 533
  The Ichthyoses: A Guide to Clinical Diagnosis, Genetic Counselling and Therapy. H.Traupe, 533
  The Look You Like: Medical Answers to 400 Questions on Skin and Hair Care. L.A.Schoen and P.Lazar, 133
Bradykinin antagonist NPC567 and human skin (Poster summary), 834
Bullous pemphigoid and malignant disease, 439
Bullous pemphigoid target antigens (Poster summary), 835
Bullous pemphigoid and target antigen specificity (Paper summary), Suppl. 37, 15

Calcitonin gene-related peptide in Raynaud's phenomenon (Paper summary), 814
Calgranulin and keratinocytes (Poster summary), 835
Candidal umbilical granuloma (Case report), Suppl. 37, 126
Capsaicin and psoriasis (Correspondence), 550
Carbon dioxide laser and port wine stain, 229
Catpox and cowpox infection (Poster summary), Suppl. 37, 67
$CD4^+/CD45\ RO^+$ lymphocytes in skin (Paper summary), 829
Child abuse and skin disease (Correspondence), 129
Chloracne, an outbreak (Paper summary), Suppl. 37, 21
Chondrodermatitis nodularis and cartilage (Paper summary), Suppl. 37, 41

Chrome dermatitis (Case report), 542
Ciprofloxacin photosensitivity, 9
Collagen type III aminopropeptide and systemic scleroderma, 1
Confocal laser immunofluorescence microscopy (Paper summary), 673
Congenital deficiency of leucocyte-adherence glycoprotein, 395
Congenital systemic mastocytosis (Case report), Suppl. 37, 70
Contact dermatitis, dominance of memory over naive T cells, 59
Contact dermatitis to latex (Paper summary), Suppl. 37, 24
Contact sensitivity to topical corticosteroids (Paper summary), Suppl. 37, 24
Contact sensitization and religious ritual (Correspondence), 846
Corticosteroid effects, age influence (Paper summary), Suppl. 37, 18
Cutaneous horns, histopathology (Paper summary), Suppl. 37, 46
Cutaneous necrosis and renal failure (Paper summary), Suppl. 37, 118
Cutaneous reactions database closure (Correspondence), 277
Cutaneous tuberculosis and serological test (Paper summary), Suppl. 37, 20
Cutaneous vasculitis (Case report), Suppl. 37, 125
Cutis laxa acquisita (Case report), 688
Cutis verticis gyrata (case report), 260
Cyclooxygenase inhibitors and herpes simplex, 375
Cyclosporin A, antiproliferative effect (Correspondence), 693
Cyclosporin A and eczematous disorders (Paper summary), Suppl. 37, 50
Cyclosporin A, fish oil and renal function (Paper summary), 535
Cyclosporin A inhibition of keratinocyte and fibroblast growth, 207
Cyclosporin A nephrotoxicity and nifedipine (Paper summary), 811
Cyclosporin A, percutaneous absorption, 631
Cyclosporin A for psoriasis (Paper summary), Suppl. 37, 43
Cyclosporin A and PUVA for psoriasis, 641
Cyclosporin A side-effects, 347
Cytokeratins and foetal keratinocytes, 707
Cytokeratins and vulval epithelium (Poster summary), Suppl. 37, 62
Cytokine levels in mycosis fungoides, 297

Dermal papilla cells and mitogenic factors (Paper summary), 815
Dermal papilla cells and testosterone (Paper summary), 816
Dermatitis herpetiformis (Case report), 262
Dermatitis herpetiformis and anti-neutrophil cytoplasmic antibodies (Paper summary), Suppl. 37, 14
Dermatophyte proteinases and fungal infections (Paper summary), 824
Dermatoses of pregnancy, comparative immunology (Poster summary), Suppl. 37, 59
Dermographic urticaria and H1 and H2 antagonists (Paper summary), 813
Dermographic urticaria and topical indomethacin (Paper summary), 813
Desmoplastic melanoma (Poster summary), Suppl. 37, 53
Diabetic foot ulcers, topical treatment, 787
Diffuse alopecia, biochemical and trichological characterization, 187
Digital collagen atrophy and Cushing's syndrome, 667
1,25-dihydroxyvitamin D_3, inhibitory effects on keratinocyte growth, 93
1,25-dihydroxyvitamin D_3 and MHC class II expression, 331
Disability index and acne (Paper summary), Suppl. 37, 21
Disability index in psoriasis, 751
Disseminated porokeratosis and craniosynostosis, 249
Dysplastic naevi, clinical features (Paper summary), Suppl. 37, 119

Eczema, thrombocytopenia and recurrent infection (Case report), Suppl. 37, 69
Eczematous plaques and subcutaneous heparin, 507
EGF receptors and proliferating cells, 49
Ehlers–Danlos syndrome and coagulation studies (Paper summary), Suppl. 37, 48
Elastic fibre damage and D-penicillamine, 305
Endothelial leukocyte adhesion molecule-1 in eczema and psoriasis (Paper summary), 828
Epidermal growth factor receptors and human epidermal tumours, 153
Epidermolysis bullosa acquisita (Case report), 677
Epidermolysis bullosa letalis, junctional (Case report), 681
Epithelioid haemangioma of bone (Case report), Suppl. 37, 85
Erosive pustular dermatosis of the leg (Correspondence), 549
Eruptive neonatal haemangiomatosis (Case report), Suppl. 37, 83
Erythema multiforme and HIV infection, 797
Erythemometry of histamine reactions (Paper summary), 821
Etretinate and teratogenicity (Correspondence), 131
Eugenol and quenching actions, 595

Factor XIIIa in malignant melanoma and Spitz naevi, 783
Familial mucocutaneous candidosis (Case report), Suppl. 37, 75
Fansidar, reactions in HIV disease (Paper summary), Suppl. 37, 28
Fatal facial ulceration (Case report), Suppl. 37, 125
Fluconazole levels in epidermis and blister fluid (Correspondence), 273
Fluorescent angiography and skin microvasculature (Poster summary), 832
Frictional properties of skin, 473
Fungi and fluorescent lectins (Poster summary), Suppl. 37, 64

Giant cell granuloma, annular elastolytic (Case report), 685
Glomus tumours and electron beam irradiation, 657
Glutenin and human connective tissue (Poster summary), Suppl. 37, 61
Glycosaminoglycans in hair follicles (Poster summary), 843
Glycosaminoglycans and human hair papilla cells (Poster summary), 842
Goldenhar syndrome (Case report), Suppl. 37, 80
Gonadotrophin releasing hormone analogue, sebum excretion and hair size (Poster summary), 840
Granulomatous cheilitis and Crohn's disease, 409

Haem arginate and variegate porphyria, 381
Hailey–Hailey disease, pathogenesis (Paper summary), 817
Hair growth *in vitro* (Paper summary), 815
H1 antagonism and the non-histamine component of antigen weals (Poster summary), Suppl. 37, 58
Heatshock protein in skin (Poster summary), 837
Hemidesmosomes and junctional epidermolysis bullosa, 137
Hereditary ichthyoses and stratum corneum lipids (Paper summary), 820
Hereditary woolly hair and ocular involvement, 523
Herlitz syndrome and BM-600 (GB3 antigen) (Paper summary), 818
Herpes simplex virus, serological response (Paper summary), 258
Histamine receptors and endothelial cells (Poster summary), 831
HLA-DR2 and Darier's disease (Correspondence), 845
Hodgkin's disease and erythema nodosum and vasculitis (Case report), Suppl. 37, 101
HTLV-1 associated cutaneous disease (Paper summary), Suppl. 37, 29
Human papillomavirus and the colposcope (Case report), 263

Ichthyoses, a re-appraisal (Paper summary), Suppl. 37, 50
Immediate contact reactions to fragrances, 595
Immune deposits in healthy skin (Paper summary), 674
Indomethacin and psoriasis (Paper summary), Suppl. 37, 43
Infantile digital fibromatosis (Case report), Suppl. 37, 92
Infantile myofibromatosis, multicentric type (Case report), 540
Infantile systemic hyalinosis and collagen (Paper summary), Suppl. 37, 36
Integrins of human epidermis, 171
Interleukin 1 and dermal dendritic cells (Paper summary), Suppl. 37, 117
Interleukin 1, effect of topical steroid (Poster summary), 841
Interleukin 1 and HIV infection, 487
Irritation by sodium lauryl sulphate, 607
Irritants and the response to allergens (Paper summary), 812
Isotretinoin, acne and inflammatory borrel disease, 653
Isotretinoin, acne and steroid metabolism (Paper summary), 816
Isotretinoin and epidermal proliferation (Paper summary), Suppl. 37, 23
Irritants and susceptibilities, 199

Juvenile xanthogranuloma (Paper summary), Suppl. 37, 122

Kallikrein-like enzyme in blister fluid (Paper summary), 819
Kallikrein in normal and psoriatic skin (Poster summary), 830
Kaposi's sarcoma cells and invasive migration, 313
Kawasaki disease (Correspondence), 413
Keratin localization in skin (Paper summary), Suppl. 37, 122
Keratin phenotype and malignant transformation of keratinocytes (Paper summary), Suppl. 37, 121
Keratinocyte allografts in dystrophic epidermolysis bullosa (Poster summary), Suppl. 37, 66
Keratinocytes and cutaneous inflammation (Paper summary), 820
Keratitis ichthyosis deafness syndrome (Case report), Suppl. 37, 77
Ki-1-positive cutaneous lymphoreticular proliferations (Paper summary), 533

Lamellar fibrosis, histogenesis (Paper summary), Suppl. 37, 121
Laminin P1 and systemic scleroderma, 1
Langerhans cells and antigen presentation, 699
Langerhans cells and foetal development (Poster summary), 841
Langerhans cell histiocytosis (Case report), 536
Langerhans cell histiocytosis and topical mechlorethamine (Paper summary), Suppl. 37, 47
Langerhans cells, sunblocks and UV damage (Poster summary), 838
Lanolin patch test, loss of positivity (Paper summary), Suppl. 37, 27
Laser Doppler velocimeter and cutaneous inflammation (Paper summary), 822
Leu-8-negative T cells in skin infiltrates, 587
Leucocyte infiltration and mediators in human skin (Poster summary), 000
Lichen planus and oral prednisolone (Correspondence), 551
Lichen planus pemphigoides and deposition of C3, C9 neoantigen and vibronectrin, 39
Limb hypertrophy (Case report), Suppl. 37, 89
Linear IgA disease and lymphoproliferative malignancy, 447
Lipocortinol antibodies and psoriasis, 569
Lipoid proteinosis (Case report), Suppl. 37, 127
LR 4005 and w-hydroxylation (Poster summary), 831
Lupus erythematosus and hereditary angioedema, 241
Lupus erythematosus and scarring alopecia (Paper summary), Suppl. 37, 13
Lupus erythematosus, sequelae (Poster summary), Suppl. 37, 55
Lymph vessels, demonstration by the extension technique, 179

Lymphangiectasis of the vulva (Case report), Suppl. 37, 92
Lymphangiomata (Case report), Suppl. 37, 93
Lymphography, isotope and the swollen limb, 29
Lymphoma, large cell follicular (Case report), 536
Lymphomatoid papulosis and lymphoma (Case report), Suppl. 37, 99

Malassezia furfur serovars A, B and C (Poster summary), 830
Malignant atrophic papulosis (Case report), Suppl. 37, 110
Malignant melanoma and cyclosporin therapy, 237
Malignant melanoma, effect of pregnancy (Paper summary), Suppl. 37, 40
Malignant melanoma, evaluation of public education campaigns, 85
Malignant melanoma, measurement of Breslow thickness (Paper summary), Suppl. 37, 123
Malignant melanoma and melanocyte atypia (Paper summary), Suppl. 37, 120
Malignant melanoma in the Netherlands (Paper summary), 259
Malignant melanoma in the over-50s age group (Poster summary), Suppl. 37, 59
Malignant melanoma and presentation (Paper summary), Suppl. 37, 39
Malignant melanoma, public education (Paper summary), Suppl. 37, 38
Malignant melanoma in Sheffield (Paper summary), Suppl. 37, 37
Mast cells and capsaicin (Paper summary), Suppl. 37, 119
Mastocytosis, diffuse cutaneous (Case report), 267
Mastocytosis, immunophenotyping and flow cytometry (Poster summary), Suppl. 37, 57
Melanin pigmentation measurement (Paper summary), Suppl. 37, 38
Melanocytic naevi and incisional biopsy, 503
Mercury patch testing in oral disease (Paper summary), Suppl. 37, 25
Micro-organisms, tolerance and autoimmunity (Paper summary), 255
Milk protein gene expression in murine sebaceous gland (Paper summary), 826
Minoxidil, inhibition of skin fibroblasts (Poster summary), 839
Mixed lymphocyte reaction in psoriasis, 325
MSH and intracellular calcium in melanoma cells (Paper summary), 827
Myelomonocytic antigens and human epidermis (Correspondence), 549

Naevoid basal cell carcinoma and radiobiological studies, 573
Nail-blanching and the finger pulp (Paper summary), Suppl. 37, 30
Nail plate, contribution of ventral nail (Paper summary), 825
Necrobiosis lipoidica (Paper summary), Suppl. 37, 47
Necrobiosis lipoidica and S100 positive dendritic cells, 765
Necrolytic migratory erythema (Case report), Suppl. 37, 107
Necrolytic migratory erythema and neuroendocrine tumour, 801
Netherton's syndrome (Case report), 269
Neutrophil zinc in psoriasis and seborrhoeic dermatitis, 319
Nodular non-Langerhans cell histiocytosis (Case report), Suppl. 37, 103

Obituary:
  Dr D.A. Birkett, 271
Onchocerciasis, classification (Paper summary), Suppl. 37, 28
Orf and milker's nodules (Paper summary), Suppl. 37, 117
Ornithine decarboxylase and sellotape stripping, 581
Out-patient dermatology referrals, 745

Pachyonychia congentia and hidradenitis suppurativa, 663
Patch testing and oral lichenoid reactions (Paper summary), Suppl. 37, 26
Patient information leaflets (Paper summary), Suppl. 37, 35
Pemphigoid and BMZ composition (Paper summary), 817
Pemphigoid gestationis (Case report), 679
Pemphigus foliaceus, endemic in Brazil, 431
Pemphigus vegetans (Case report), Suppl. 37, 112
Pemphigus vulgaris, conjunctival involvement, 615
Pentoxifylline and fibroblast proliferation, 339
Pentoxifylline and metastatic melanoma cells (Paper summary), 828
Peptide histidine methionine in eccrine sweat glands, 65
Peptidergic neurotransmitter innervation and eccrine sweat glands (Poster summary), Suppl. 37, 56
Persistent bite reactions and photochemotherapy (Correspondence), 693
Phenytoin and would healing (Paper summary), Suppl. 37, 17
Phospholipase C activity in atopics (Poster summary), 838
Phospholipase A2 and HDMNKVLDL (Paper summary), 819
Phospholipase A2, inhibition by polyunsaturated fatty acids (Poster summary), Suppl. 37, 56
Pityriasis rubra pilaris and Indians (Correspondence), 692
Plant allergens (Poster summary), Suppl. 37, 61
Plantar fibromatosis (Poster summary), Suppl. 37, 62
Plaque clearance and anthralin therapy (Poster summary), 840
Porokeratosis disseminata and cutaneous amyloidosis (Case report), 683
Prenatal diagnosis and genetic skin disease (Paper summary), Suppl. 37, 37
Pretibial epidermolysis bullosa (Poster summary), Suppl. 37, 53
Proteus syndrome (Case report), 538
Pruritus and anxiety and depression, 769
Pruritus vulvae and contact hypersensitivity (Paper summary), Suppl. 37, 26

Pseudo-Degos disease (Case report), Suppl. 37, 111
Pseudolymphoma and malignant B-cell lymphoma (Case report), Suppl. 37, 95
Pseudoporphyria and nalidixic acid (Correspondence), 548
Psoriasis, changes with calcipotriol and betamethasone treatment (Poster summary), 837
Psoriasis and consultation audit, 99
Psoriasis day care centre (Paper summary), 534
Psoriasis, fibroblast-keratinocyte interactions, 467
Psoriasis and fumaric acid therapy (Paper summary), 534
PUVA-associated squamous cell carcinoma (Poster summary), Suppl. 37, 65
PUVA and chronic actinic dermatitis (Correspondence), 273
PUVA erythema and psoralen levels (Paper summary), 823
PUVA, long-term effects on fibroblasts, 621
PUVA with 325, 352 and 370 nm lamps (Paper summary), 823
Pyoderma gangrenosum and vancomycin and mezlocillin, 389

RAR expression in keratinocytes and fibroblasts (Paper summary), 827
Ras p21 and differentiating cells, 49
Raynaud's phenomenon and iloprost infusion (Paper summary), Suppl. 37, 49
Retinoic acid, effect on interleukin 1 in epidermis, 283
Retinoic acid receptors and S91 melanoma cells (Paper summary), 827
Retinoic acid receptors in sebaceous glands (Poster summary), 836
Rosacea, presentation patterns and response to therapy (Poster summary), Suppl. 37, 66

Sarcoid-like reaction (Case report), Suppl. 37, 106
Schopf syndrome (Case report), 543
Sclerodermatomyositis, effect of intravenous immunoglobulin (Correspondence), 546
Sclerosing sweat duct carcinoma (Case report), Suppl. 37, 127
Seborrhoeic warts, cell kinetics (Poster summary), 836
Silica-associated systemic sclerosis, 725
Sjögren–Larrson syndrome (Paper summary), Suppl. 37, 16
Skin colour, measurements and perception, 77
Smooth muscle hamartoma (Case report), 539
Sneddon's syndrome and haemostasis (Paper summary), Suppl. 37, 35
Solar keratosis, prevalence (Paper summary), Suppl. 37, 40
Spindle cell naevus, 757
Squamous cell carcinoma and hidradenitis suppurativa, 527
Squamous cell carcinoma of the nail bed, 215
SSA/Ro antigen and risk of lupus erythematosus, 223
Stem cell glycoprotein and basal keratinocytes (Paper summary), 818
Streptococcal cellulitis, perianal and penile, 793
Strimmer dermatitis (Poster summary), Suppl. 37, 63
Sulphamethoxypyridazine and obliterative brochiolitis and alveolitis (Correspondence), 125
Sunblocks, Langerhans cells and UV damage (Poster summary), 832
Systemic scleroderma and autoantibodies, 421

T-cell leukaemia/lymphoma (Case report), Suppl. 37, 100
T-cell lymphoma and follicular B-cell lymphoma (Case report), 266
T-cell lymphoma and panniculitis, 649
T-lymphocytes and psoriasis (Paper summary), 256
Terbinafine and nail matrix kinetics (Paper summary), Suppl. 37, 31
Terbinafine and nail penetration (Paper summary), 825
Terbinafine and onychomycoses, 481
Terbinafine and onychomycosis (Paper summary), Suppl. 37, 31
Terbinafine and tinea infection (Paper summary), Suppl. 37, 30
Terfenadine and anthralin inflammation (Paper summary), 812
Tixcocortal pivalate and hydrocortisone sensitivity (Paper summary), Suppl. 37, 25
Tonofilament clumping and Dowling–Meara epidermolysis bullosa simplex (Poster summary), 834
Topical PUVA in palmoplantar pustulosis (Paper summary), Suppl. 37, 44
Toxic erythemas (Paper summary), Suppl. 37, 18
Toxic pustuloderma, 119
Trichophyton rubrum factor inhibition of blastogenesis (Paper summary), 826
Tyrosinase expression in follicular melanocytes (Poster summary), 842
Tyrosinase expression and B16 F10 mouse melanoma (Poster summary), 832

Ultrasound and nail thickness, 365
Unilesional mycosis fungoides (Paper summary), Suppl. 37, 123
Urticaria and anxiety and depression, 769
Urticaria and cancer, 453
Urticaria and the common stinging nettle (Poster summary), Suppl. 37, 62
Urticaria and a histamine releasing factor (Paper summary), Suppl. 37, 45
Urticaria pigmentosa and topical steroids (Paper summary), Suppl. 37, 45
Urticarial vasculitis and IgM gammopathy, 113
UV blocking lenses for PUVA, 775
UV lenses for PUVA (Poster summary), Suppl. 37, 58
UVB erythema and topical solvents (Paper summary), 821
UVB and UVB sunscreen protection (Paper summary), 822

Vapour-permeable wound dressing (Paper summary), 676
Varicella bullosa (Correspondence), 846
Vascular responses and topical steroid (Paper summary), 814
Vasoactive intestinal peptide in eccrine sweat glands, 65
Venous stasis (Correspondence), 274

Venous ulcers and compression bandaging (Poster summary), Suppl. 37, 54
Venous ulceration and lymphatic drainage (Paper summary), Suppl. 37, 33
Vitiligo induced by diphencyprone (Correspondence), 415
Vitamin D_3 analogue (MC903), effect on DNA content and Ks8.12 binding in psoriatic lesion, 291
Von Willebrand factor antigen, immunoradiometric assay, 21

Warts and response to current treatment (Paper summary), Suppl. 37, 19
White piedra and synergistic infection, 355

X-linked ichthyosis and steroid sulphatase activity (Correspondence), 547
Xanthoma dissemination and histiocytosis X (Paper summary), Suppl. 37, 16
Xanthomatosis, subcutaneous, 107
Xeroderma pigmentosum and cell survival after UVC (Paper summary), 824

Yaws in Sumatra (Paper summary), 258
Yellow nail syndrome and lymphoscintography (Paper summary), Suppl. 37, 32

AUTHORS

Abiose, A. *see* Murdoch, M.E.
Aegidius, J. *see* Brandrup, F.
Aiba, S. *see* Terui, T.
Aitchison, T. *see* Tillman, D.M.
Aitchison, T.C. *see* Williams, R.E.A.
Ala-Kokko, L. *see* Oikarinen, A.
Alaybeyi, F. *see* Kürkçüoğlu, N.
Aldridge, R.D. *see* Vestey, J.P.
Alexander, L. *see* Wilkinson, S.M.
Allen, B.R. *see* Brown, R.T.
Allen, B.R. *see* Sowden, J.M.
Allen, C. *see* Wilkinson, S.M.
Allen, J. *see* Eady, R.A.J.
Allen, J. *see* Frith, P.
Allen, J. *see* Venning, V.
Allen, M.H. *see* Barker, J.N.W.N.
Allen, M.H. *see* Groves, R.W.
Allen, M.H. *see* Markey, A.C.
Allenby, C.F. *see* Safford, R.J.
Amagai, M. *see* Kikuchi, A.
Amon, E.U. *see* Oliver, F.
Anegg, B. *see* Petzelbauer, P.
Anstey, A., Beechey-Newman, N., Gowers, L. and Molloy, H. A case of candidal umbilical granuloma (Case report), Suppl. 37, 126
Anstey, A.V., Wilkinson, J.D. and Lord, P. Squamous cell carcinoma complicating hidrandenitis suppurativa, 527
Anstey, A.V., Winter, M. and Pope, F.M. Platelet and coagulation studies in Ehlers Danlos syndrome (Paper summary), Suppl. 37, 48
Anstey, A.V. *see* Arlett, C.F.
Apelqvist, J., Larsson, J. and Stenstrom A. Topical treatment of necrotic foot ulcers in diabetic patients: a comparative trial of DuoDerm and MeZinc, 787
Archer, C.B., Sharvill, D.E. and Smith N.P. Subcutaneous xanthomatosis, 107
Archer, C.B. *see* Reynolds, N.J.
Arlett, C.F., Harcourt, S.A., Cole, J. and Anstey, A.V. Cell survival after UVC treatment of fibroblasts, T-lymphocytes in G_0 and dividing lymphocyte lines from normal individuals and an excision defective xeroderma pigmentosusm patient (Paper summary), 824
Arlett, C.F. *see* Newton, J.A.
Armstrong E.P. *see* Eedy, D.J.
Arnold, W.P., Van Hooijdonk, C.A.E.M., Mier, P.D. and Van De Kerkhof, P.C.M. Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin, 581
Ashbee, H.R., Cunningham, A.C., Ingham, E., Holland, K.T., Cunliffe, W.J. and Gowland, G. Carriage, serology and immunological responses to *Malassezia furfur* serovars A, B and C (Poster summary), 830
Ashcroft, A.T.T. *see* Taylor, M.
Ashworth, J., White, I.R., Rycroft, R.J.G. and Cronin, E. Contact sensitivity to topical corticosteroids (Paper summary), Suppl. 37, 24
Atherton, D.J. *see* Glover, M.T.
Atherton, D.J. *see* Sheehan, M.P.
Aurelian, L. *see* Wachsman, M.

Bacon, K.B. *see* Lawlor, F.
Bacon, K.B. *see* Ross, J.S.
Baker, B.S. *see* Powles, A.V.
Baker, H. *see* Salisbury, J.A.
Bal, W. *see* Burchill, S.A.
Ballardie, F.W. *see* Edwards, B.D.
Balsiger, F. *see* Merot, Y.
Barbenel, J.C. *see* Cherry, G.M.
Barkema, L.W. *see* De Jong, M.C.J.M.
Barker, J.N.W.N., Allen, M.H., Griffiths, C.E.M., Nickoloff, B.J. and MacDonald, D.M. Expression of the myelomonocytic antigens L1 and CD36 in human epidermis (Correspondence), 548
Barker, J.N.W.N., Mitra, R.S., Griffiths, C.E.M., Dixit, V.M. and Nickoloff, B.J. Potential role for keratinocytes as initiators of cutaneous inflammation (Paper summary), 820
Barker, J.N.W.N. *see* Groves, R.W.
Barkley, A. and Shall, L. Erythemometry of low level cutaneous histamine reactions (Paper summary), 821
Barnes, L. *see* Jackson, J.
Barnetson, R.St.C. *see* Kemmett, D.
Barton, S.P. *see* Marks, R.
Barton, S.P. *see* Mourad, M.M.
Basketter, D.A. *see* Safford, R.J.
Bates, D. *see* Bull, R.H.
Baverstock, J. *see* Mourad, M.M.
Beacham, B.E. and Kurgansky, D. Persistent bite reactions responsive to photochemotherapy (Correspondence), 693
Beechey-Newman, N. *see* Anstey, A.
Beljaards, R.C. Ki-1-positive cutaneous lymphoreticular proliferations (Paper summary), 533
Beljaards, R.C. Anetodermia and borreliosis (Case report), 541
Beljaards, R.C. The proteus syndrome (Case report), 538
Benner, K. *see* Prens, E.P.
Bennett, D.C. *see* Burchill, S.A.
Benton, E.C. *see* Harris, D.W.S.
Bergfeld, W.F. *see* Guitart, J.
Berman, B. and Duncan, M.R. Pentoxifylline inhibits the proliferation of human fibroblasts derived from keloid scleroderma and morphoea skin and their production of collagen, glycosaminoglycans and fibronectin, 339
Berry, P.J. *see* Potter, M.N.

Berth-Jones, J. and Hutchinson, P.E. Response rates to current treatment of common warts and evaluation of continued cryotherapy and inosine pranobex for treatment failures (Paper summary), Suppl. 37, 19
Besley, G.T. *see* Judge, M.R.
Beutner, E.H. *see* Blaszczyk, M.
Bhogal, B. *see* Robles Martinez, W.
Bhoola, K.D., Poblete, M.T., Reynolds, N.J., Figueros, C.D., Burton, J.L. and Muller-Esterl, W. Cellular localization of tissue kallikrein and kininogen in the normal and psoriatic human skin (Poster summary), 830
Bhoola, K.D. *see* Nicholls, B.M.
Bilo, H.J.E. *see* Stoof, T.J.
Bilsland, D. and Douglas, W.S. Sunbed pseudoporphyria induced by nalidixic acid (Correspondence), 547
Bircher, A.J., Fluckiger, R. and Buchner, S.A. Eczematous infiltrated plaques to subcutaneous heparin: a type IV allergic reaction, 507
Bird, C.R. *see* Ross, J.S.
Bittiner, S.B., Variend, S. and Bleehen, S.S. A histopathological review of 21 cases of juvenile xanthogranuloma (Paper summary), Suppl. 37, 122
Black, D. and Marks, R. Age influence on topical corticosteroid effects on skin (Paper summary), Suppl. 37, 18
Black, M.M. *see* Cerio, R.
Black, M.M. *see* Fenton, D.A.
Black, M.M. *see* Kelly, S.E.
Blaszczyk, M., Jarzabek-Chorzelska, M., Jabłońska, S., Chorzelski, T., Kołacińska-Strasz, Z., Beutner, E.H. and Kumar, V. Autoantibodies to nucleolar antigens in systemic scleroderma: clinical correlations, 421
Bleehen, S.S. *see* Bittiner, S.B.
Bleehen, S.S. *see* MacNeil, S.
Bleehen, S.S. *see* McDonagh, A.J.G.
Blindeman, L. *see* Tio, T.T.
Blindeman, L. Use of the colposcope ('peniscopy') in subclinical human papillomavirus (HPV) infection of the penis (Case report), 263
Blount, M.A. *see* Watson, L.P.
Bodemar, C., Teillac, D., Le Bourgeois, M., Wechsler, B. and De Prost, Y. Efficacy of intravenous immunoglobulins in sclerodermatomyositis (Correspondence), 545
Bonduelle, M. *see* Rademaker, M.
Boorsma, D.M. *see* Rijlaarsdam, J.U.
Borradori, L., Rybojad, M., Puissant, A., Dallot, A., Verola, O. and Morel, P. Urticarial vasculitis associated with a monoclonal IgM gammopathy: Schnitzler's syndrome, 113
Bos, J.D. *see* De Rie, M.A.
Boyle, J. Lymphomatoid papulosis progressing to high-grade lymphoma (Case report), Suppl. 37, 99
Boyle, J. Nodular non-Langerhans cell histiocytosis (Case report), Suppl. 37, 103
Boyle, J. Sarcoid-like reaction: case for diagnosis (Case report), Suppl. 37, 106
Bradfield, J.W. *see* Reynolds, N.J.
Brakenhoff, G.J. *see* De Jong, M.C.J.M.
Brandrup, F., Petri, J. and Aegidius, J. Acantholytic lesions in the vagina (Correspondence), 691
Bransbury, D. *see* Carmichael, A.J.
Brasch, J. *see* Sterry, W.
Braun-Falco, O. *see* Schirren, C.G.
Breathnach, A. *see* Oliver, F.
Breathnach, S, *see* Hintner, H.
Brett, J.V. *see* Du Vivier, A.W.P.
Brickell, P.M., Maschio, A., Katz, D., Bunker, C.B. and Craig, R.K. Expression of milk protein genes in the sebaceous glands of transgenic and normal mice during lactation (Paper summary), 826
Broadbent, V. *see* Sheehan, M.P.
Brown, R.T., Sowden, J.M. and Allen, B.R. LR4005 inhibits the w-hydroxylation of 12(S)-HETE by PMNLs (Poster summary), 831
Bruen, S. *see* Sterry, W.
Bruin, P. *see* Jonkman, M.F.
Bruins, F.G. and Bruynzeel, D.P. Chrome dermatitis (Case report), 542
Bruins, S. *see* De Jong, M.C.J.M.
Bruynzeel, D.P. *see* Bruins, F.G.
Buchanan, K.D. *see* Eedy, D.J.
Buchner, S.A. *see* Bircher, A.J.
Bufalino, R. *see* MacKie, R.M.
Buffey, J. *see* Friedmann, P.S.
Buffey, J. *see* MacNeil, S.
Buitenwerf, J. *see* Van Ulsen, J.
Bull, H.A., Courtney, P.C. and Dowd, P.M. Characterisation of histamine receptors on human endothelial cells (Poster summary), 831
Bull, H.A. *see* Bunker, C.B.
Bull, H.A. *see* Rustin, M.H.A.
Bull, R.H., Bates, D. and Mortimer, P.S., Intravital native and fluorescent angiography for the assessment of the skin microvasculature: a preliminary report (Poster summary), 832
Bull, R.H., Mortimer, P.S., Gane, J. and Joseph, A.G.A. Abnormal lymph drainage in patients with chronic venous leg ulcers (Paper summary), Suppl. 37, 33
Bull, R.H., Ryan, J.F., Cook, M.G. Fatal facial ulceration (Case report), Suppl. 37, 125
Bull, R.H., Staughton, R.C.D. Increased risk of cutaneous reactions to Fansidar in HIV disease (Paper summary), Suppl. 37, 28
Bull, R. *see* Fenton, D.A.
Bunker, C.B., Cerio, R., Bull, H.A., Evans, J., Foreman, J.C. and Dowd, P.M. Mast cells after capsaicin application in normal human skin (Paper summary), Suppl. 37, 119
Bunker, C.B., Foreman, J.C. and Dowd, P.M. Topical steroid and the vascular responses to intradermal neuropeptides, histamine and compound 48/80 (Paper summary), 814
Bunker, C.B., Terenghi, G., Springall, D.R., Polak, J.M. and Dowd, P.M. Calcitonin gene-related peptide and endothelin in digital skin in Raynaud's phenomenon (Paper summary), 814
Bunker, C.B. *see* Brickell, P.M.
Burchill, S.A., Bal, W., Hill, S.E., MacNeil, S., Bennett, D.C. and Thody, A.J. Tyrosinase ex-

pression in a new amelanotic subline of the B16 F10 mouse melanoma (Paper summary), 832
Burchill, S.A., Ito, S. and Thody, A.J. Tyrosinase expression in human hair follicular melanocytes and its relationship to the synthesis of eumelanin and phaeomelanin (Paper summary), 842
Burge, S.M., Cederholm-Williams, S.A., Leigh, I.M. and Garrod, D.R. The pathogenesis of Hailey-Hailey disease: clinical, immunopathological and tissue culture studies (Paper summary), 817
Burge, S.M. *see* Dalziel, K.L.
Burge, S.M. *see* De Berker, D.
Burge, S.M. *see* Venning, V.
Burge, S.M. *see* Wilson, C.L.
Burnett, J.W. *see* Wachsman, M.
Burrows, N.P. and Russell Jones, R. Cutaneous necrosis in chronic renal failure (Paper summary), Suppl. 37, 118
Burrows, N.P. and Russell Jones, R. Cutaneous vasculitis following administration of anisoylated plasminogen streptokinase activator complex (APSAC) (Case report), Suppl. 37, 125
Burrows, N.P. and Russell Jones, R. Pretibial epidermolysis bullosa: a report of three families (Poster summary), Suppl. 37, 53
Burton, J.L. Is 'venous stasis' outmoded? Reply (Correspondence), 276
Burton, J.L. *see* Bhoola, K.D.
Burton, J.L. *see* Nicholls, B.M.
Burton, J.L. *see* Reynolds, N.J.
Burton, P.A. *see* Kennedy, C.T.C.
Byrne, J.P.H. *see* Wilkinson, S.M.

Cameron, J. *see* Cherry, G.M.
Camp, R.D.R. *see* Lawlor, F.
Camp, R.D.R. *see* McFadden, J.P.
Camp, R.D.R. *see* Ross, J.S.
Carmichael, A.J., Foulds, I.S. and Bransbury, D. Loss of lanolin patch test positivity (Paper summary), Suppl. 37, 27
Carmichael, A.J. and Foulds, I.S. Sensitization as a result of a religious ritual (Correspondence), 846
Cartwright, P.H., Ilderton, E., Wilkinson, S.M. and Yardley, H.J. Phospholipase A_2 activity is inhibited in intact lesion-free psoriatic epidermis by the nonapeptide HDMNKVLDL which has lipocortin-like activity (Paper summary), 819
Cartwright, P.H. *see* Wilkinson, S.M.
Cartwright, P.H., Wilkinson, S.M. and English, J.S.C. Tixocortal pivalate is a marker for hydrocortisone sensitivity (Paper summary), Suppl. 37, 25
Casparie, A.F. *see* Van Everdingen, J.J.
Cassella, J.P. *see* Ishida-Yamamoto, A.
Cassella, J-P, *see* Schofield, O.M.V.
Catty, D. *see* Duhra, P.
Cawood, L. *see* McDonagh, A.J.G.
Cederholm-Williams, S.A. *see* Burge, S.M.
Cerio, R., Smith, N.P. and Wilson Jones, E. Xanthoma dissemination and histiocytosis X: rare dermatoses easily confused (Paper summary), Suppl. 37, 16
Cerio, R., Sonnex, T.S., Smith, N.P. and Wilson Jones, E. Desmoplastic melanoma: a rare form of malignant melanoma that is usually misdiagnosed and mismanaged (Poster summary), Suppl. 37, 53
Cerio, R. and Black, M.M. Regressing atypical histiocytosis and lymphomatoid papulosis: variants of the same disorder?, 515
Cerio, R. and Wilson Jones, E. The histogenesis of lamellar fibrosis: a new concept (Paper summary), Suppl. 37, 121
Cerio, R. *see* Bunker, C.B.
Cerio, R. *see* McFadden, J.P.
Cerio, R. *see* Ross, J.S.
Chagnon, J.L. *see* Machet, L.
Chalmers, R.J. *see* Edwards, B.D.
Chambers, B., Milligan, A. and Fletcher, A. Epidermal dendritic S100 positive cells in necrobioisis lipoidica and granuloma annulare, 765
Chapman, S.J., Leigh, I.M., Tidman, M.J. and Eady, R.A.J. Abnormal expression of hemidesmosome-like structures by junctional epidermolysis bullosa keratinocytes *in vitro*, 137
Chapman, S.J. *see* Ishida-Yamamoto, A.
Chatellard Gruaz, D., Didierjean, L., Gumowski-Sunek, D. and Saurat, J-H. Effect of topical retinoic acid on the interleukin 1 alpha and beta immunoreactive pool in normal human epidermis, 283
Cherry, C.A. *see* Cherry, G.M.
Cherry, G.W., Sockalingen, S., Clay, C.S., Cameron, J., Cherry, C.A., Barbenel, J.C., Ryan, T.J., Queen, D. and Frank, M. Evaluation of a unique new compression bandage in the treatment of venous ulcers (Poster summary), Suppl. 37, 54
Chorzelski, T. *see* Błaszczyk, M.
Chu, A. *see* Devereux, J.
Chu, A. *see* Morris, J.
Chu, A. *see* Sheehan, M.
Clay, C.S. *see* Cherry, G.W.
Cobby, M. *see* Reynolds, N.J.
Coenen, J.M.F.H. *see* Jonkman, M.F.
Coenraads, P.J. *see* Tupker, R.A.
Coenraads, P.J. *see* Van Voorst Vader, P.C.
Cole, J. *see* Arlett, C.F.
Cole, J. *see* Newton, J.A.
Collins, C. *see* Sansom, J.
Colver, G.B., Harris, D.W.S. and Tidman, M.J. Skin diseases that may mimic child abuse (Correspondence), 129
Cominos, B. *see* Hindson, C.
Cook, M.G. *see* Bull, R.H.
Cook, M.G. *see* Curley, R.K.
Cook, M.G. *see* Fallowfield, M.E.
Cook, P. *see* Kennedy, C.T.C.
Cooperative Group for Fogo Selvagem Research, *see* Empinotti, J.C.
Cotterill, J.A. and Lanigan, S.W. Erosive pustular dermatosis of the leg—a definition (Correspondence), 548
Cotterill, J.A. *see* Lanigan, S.W.
Cotterill, J.A. *see* Sheehan-Dare, R.A.
Cotton, D.W.K. *see* Denton, K.J.
Cotton, D.W.K. *see* McDonagh, A.J.G.
Coulson, I.H. *see* Mallett, R.B.
Courtney, P.C. *see* Bull, H.A.

Coutts, J.R.T. *see* Rademaker, M.

Craig, R.K. *see* Brickell, P.M.

Cronin, E. *see* Ashworth, J.

Cua, A.B., Wilhelm, K-P. and Maibach, H.I. Cutaneous sodium lauryl sulphate irritation potential: age and regional variability, 607

Cua, A.B., Wilhelm, K-P. and Maibach, H.I. Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss, 473

Cunliffe, W.J. *see* Ashbee, H.R.

Cunliffe, W.J. *see* Layton, A.M.

Cunliffe, W.J. *see* Nutbrown, N.

Cunningham, A.C. *see* Ashbee, H.R.

Curley, R.K., Fallowfield, M.E., Cook, M.G. and Marsden, R.A. The clinical features of dysplastic naevi: inter-observer agreement and correlation with histology (Paper summary), Suppl. 37, 119

Curley, R.K., Fallowfield, M.E., Cook, M.G. and Marsden, R.A. Effect of incisional biopsy on subsequent histology of melanocytic naevi, 503

Da Cunha Bang, F. *see* Oxholm, A.

Dahlback, B. *see* Hintner, H.

Dahlback, K. *see* Hintner, H.

Dallot, A. *see* Barradori, L.

Daly, A. *see* Redfern, C.P.F.

Dalziel, K.L., Burge, S.M., Frith, P.A., Ryan, T.J. and Mowat, A. Elastic fibre damage induced by low-dose D-penicillamine, 305

Dalziel, K. *see* Venning, V.

D'Arcy, S. *see* Duhra, P.

David, M. *see* Hodak, E.

David, T.J. *see* Judge, M.R.

Dawber, R.P.R. *see* Wilson, C.L.

Dean, D. *see* Wilson, C.L.

De Berker, D., Burge, S.M., Dissanayake, M. and Wojnarowska, F. The sequelae of chronic cutaneous lupus erythematosus (Paper summary), Suppl. 37, 55

De Haan, P. *see* Rijlaarsdam, J.U.

De Jong, M.C.J.M., Barkema, L.W., Bruins, S., Van Luijk, W.H.J. and Zwierstra, E.P. Immune deposits in the skin of healthy children (Paper summary), 674

De Jong, M.C.J.M., Bruins, S., Heeres, K., Brakenhoff, G.J. and Leigh, I.M. Confocal laser immunofluorescence microscopy: a future tool in the differential diagnosis of subepidermal (immuno)bullous dermatoses (Paper summary), 673

De Jong, M.C.J.M., Jonkman, M.F., Van Voorst Vader, P.C. and Hollema, H. Epidermolysis bullosa acquisita (Case report), 677

De Jong, M.C.J.M., Jonkman, M.F., Van Voorst Vader, P.C. and Hollema, H. Pemphigoid gestationis (Case report), 679

De Jong, E.C.J.M., Meijer, P., Van Voorst Vader, P.C. and Hollema, H. Junctional epidermolysis bullosa letalis (Case report), 681

De Jong, M.C.J.M. *see* De Mare, S.

De Jong, M.C.J.M. *see* Keukens, F.M.C.

De Jong, M.C.J.M. *see* Meijer, P.

De Jong, M.C.J.M. *see* Van Gerven, C.C.M.

De Jong, M.C.J.M. *see* Van Voorst Vader, P.C.

De Koning, J. *see* Tazelaar, D.J.

Delaney, T.J. and Uff, J.S. Necrolytic migratory erythema: apparent response to oral omega-3 (marine) essential fatty acids (Case report), Suppl. 37, 107

De Mare, S., De Jong, M.C.J.M. and Van De Kerkhof, P.C.M. DNA content and Ks8.12 binding of the psoriatic lesion during treatment with the vitamin D3 analogue MC903 and betamethasone, 291

Denton, K.J., Cotton, D.W.K., Wright, A. and Hird, P. Factor XIIIa in nodular malignant melanoma and Spitz naevi, 783

De Prost, Y. *see* Bodemar, C.

De Rie, M.A., Meinardi, M.M.H.M. and Bos, J.D. Analysis of side-effects of medium and low-dose cyclosporin maintenance therapy in psoriasis, 347

Devereux, J., Morris, J., Walker, S. and Chu, A. The effect of commercial sun-blocks in preventing UV damage to Langerhans cell function *in vivo* (Poster summary), 833

Devereux, J. *see* Morris, J.

Diaz, L.A. *see* Empinotti, J.C.

Diderjean, L. *see* Chatellard Gruaz, D.

Diffey, B.L. and Farr, P.M. Sunscreen protection against UVB and UVA: an *in vitro* and *in-vivo* comparison (Paper summary), 822

Diffey, B.L. *see* Farr, P.M.

Diffey, B.L. *see* Langtry, J.A.A.

Diffey, B.L. *see* McLelland, J.

Dissanayake, M. *see* De Berker, D.

Dixit, V.M. *see* Barker, J.N.W.N.

Djakovic, Z. *see* Stevanovic, D.V.

Dobson, J. *see* MacNeil, S.

Docherty, V.R., Forsyth, A. and MacKie, R.M. Pruritus vulvae: a manifestation of contact hypersensitivity (Paper summary), Suppl. 37, 26

Dohi, K. *see* Miyagawa, S.

Douglas, W.S. *see* Bilsland, D.

Dover, J.S. *see* Kang, S.

Dowd, P.M. *see* Bull, H.A.

Dowd, P.M. *see* Bunker, C.B.

Dowd, P.M. *see* Rustin, M.H.A.

Downey, A. *see* Hindson, C.

Dreno, B., Milpied, B., Dutartre, H. and Litoux, P. Epidermal interleukin 1 in normal skin of patients with HIV infection, 487

Duhra, P., Catty, D., Jassim, D. and D'Arcy, S. A serological test for rapid diagnosis of cutaneous tuberculosis (Paper summary), Suppl. 37, 20

Duhra, P. and Foulds, I.S. Persistent vitiligo induced by diphencyprone (Correspondence), 415

Duhra, P. and Ilchyshyn, A. Perianal streptococcal cellulitis with penile involvement, 793

Duhra, P., Hodgson, C., Ilchyshyn, A. and Porter, D.I. Bath PUVA for psoriasis: the West Midlands experience (Paper summary), Suppl. 37, 42

Duhra, P., Holmes, J. and Porter, D.I. Discoid lupus erythematosus associated with hereditary angioneurotic oedema, 241

Dumas, A.M. *see* Van Ulsen, J.

Duncan, J.I., Payne, S.N.L., Winfield, A.J., Ormerod,

A.D. and Thomson, A.W. Enhanced percutaneous absorption of a novel topical cyclosporin A formulation and assessment of its immunosuppressive activity, 631
Duncan, A. *see* Kennedy, C.T.C.
Duncan, M.R. *see* Berman, B.
Dutartre, H. *see* Dreno, B.
Du Vivier, A.W.P., Williams, H.C., Brett, J.V. and Higgins, E.M. How do malignant melanomas present and does this correlate with the 7-point check list? (Paper summary), Suppl. 37, 39
Du Vivier, A.W.P. *see* Williams, H.C.
Dykes, P.J. *see* Finlay, A.Y.
Dykes, P.J. *see* Nazmi, M.N.
Dykes, P.J. *see* Samdani, A.J.
Dykes, P.J. *see* Vijayasingam, S.M.
Dykes, P.J., Thomas, R. and Finlay, A.Y. Determination of terbinafine in nail samples during systemic treatment for onychomycoses, 481

Ead, R.D. *see* Kellett, J.K.
Eady, R.A.J., Gunner, D.B., Lake, B.D., Heagerty, A.H.M., Schofield, A.M.V., Allen, J., MacKinnon, P., Nicolaides, K.H. and Rodeck, C.H. Prenatal diagnosis of genetic skin disease using foetal skin biopsy: 10 years' experience (Paper summary), Suppl. 37, 37
Eady, R.A.J. *see* Chapman, S.J.
Eady, R.A.J. *see* Ishida-Yamamoto, A.
Eady, R.A.J. *see* Parker, S.C.
Eady, R.A.J. *see* Schofield, O.M.V.
Eady, R.A.J. *see* Verrando, P.
Eberhard Klein, C., Steinmayer, T., Mattes, J.M., Kaufmann, R. and Weber, L. Integrins of normal human epidermis: differential expression, synthesis and molecular structure, 171
Edward, M. and MacKie, R.M. Pentoxifylline enhances lung colonization and glycosaminoglycan synthesis by metastatic melanoma cells (Paper summary), 828
Edward, M. *see* Grant, A.W.
Edwards, B.D., Testa, H.J., Lawson, R.S., Chalmers, R.J., O'Driscoll, J. and Ballardie, F.W. Nifedipine modulates the haemodynamics and vasoactive regulation of cyclosporin nephrotoxicity in the normal human kidney (Paper summary), 811
Edwards, C., Heggie, R. and Marks, R. In vivo measurement of melanin pigmentation (Paper summary), Suppl. 37, 38
Edwards, C. *see* Finlay, A.Y.
Eedy, D.J., Shaw, C., Armstrong, E.P., Johnston, C.F. and Buchanan, K.D. Vasoactive intestinal peptide (VIP) and peptide histidine methionine (PHM) in human eccrine sweat glands: demonstration of innervation, specific binding sites and presence in secretions, 65
Eedy, D.J., Johnston, C.F., Shaw, C. and Buchanan, K.D. Peptidergic neurotransmitter innervation of the eccrine sweat gland and their presence in sweat secretions (Poster summary), Suppl. 37, 56
Eklund, G. *see* Lindelof, B.
Ellis, J.P. *see* Neill, S.M.
Ellis, J.P. Wegener's granulomatosis presenting with blepharo-conjunctivitis (Case report), Suppl. 37, 104
Ellner, K.M., McBride, M.E., Kalter, D.C., Tschen, J.A. and Wolf, J.E., Jr. White piedra: evidence for a synergistic infection, 355
Empinotti, J.C., Diaz, L.A., Martins, C.R., Rivitti, E.A., Sampaio, S.A.P., Lombardi, C., Sanches, J.A. and Cooperative Group for Fogo Selvagem Research. Endemic pemphigus foliaceus in western Parana, Brazil (1976–1988), 431
Engelkens, H.J.H., Jubianto, J., Noordhoek, G.T., Van Embden, J., Van Der Sluis, J.J., Vuzevski, V.D. and Stolz, E. Yaws in Sumatra, Indonesia, 1988 (Paper summary), 258
English, J.S.C. *see* Cartwright, P.H.
Essex, T. *see* Quinn, A.G.
Evans, E.G.V. *see* Harland, C.C.
Evans, E.G.C. *see* White, J.E.
Evans, J. *see* Bunker, C.B.

Fallowfield, M.E. and Cook, M.G. Melanocyte atypia and melanoma (Paper summary), Suppl. 37, 120
Fallowfield, M.E. *see* Curley, R.K.
Farr, P.M., Diffey, B.L., Higgins, E.M. and Matthews, J.N.S. PUVA treatment of psoriasis comparing lamps with peak emission at 325, 352 or 370 nm (Paper summary), 823
Farr, P.M. *see* Diffey, B.L.
Farr, P.M. *see* McLelland, J.
Farr, P.M. *see* Quinn, A.G.
Fenton, D.A., Bull, R., Gane, J. and Mortimer, P.S. Abnormal lymphatic function assessed by quantitative lymphoscintography in yellow nail syndrome (Paper summary), Suppl. 37, 32
Fenton, D.A., Tobin, D.J., Black, M.M. and Kendal, M.D. Incomplete melanogensis with melanocyte abnormalities in acute alopecia areata (Paper summary), Suppl. 37, 34
Ferguson, J. and Johnson, B.E. Ciprofloxacin-induced photosensitivity: *in vitro* and *in vivo* studies, 9
Feuerman, P. *see* Hodak, E.
Figueros, C.D. *see* Bhoola, K.D.
Fincham, N.J. *see* Ross, J.S.
Finlay, A.Y., Khan, G.K., Luscombe, D.K. and Salek, M.S. Validation of Sickness Impact Profile and Psoriasis Disability Index in psoriasis, 751
Finlay, A.Y., Lever, L., Thomas, R. and Dykes, P.J. Nail matrix kinetics of systemic terbinafine in onychomycosis (Paper summary), Suppl. 37, 31
Finlay, A.Y., Western, B. and Edwards, C. Ultrasound velocity in human fingernail and efffects of hydration: validation of *in vivo* nail thickness measurements, 365
Finlay, A.Y. *see* Dykes, P.J.
Finlay, A.Y. *see* Motley, R.J.
Finlay, A.Y. *see* Shuttleworth, D.
Finnen, M.J. and Lovell, C.R. Inhibition of human epidermal phospholipase A2 by polyunsaturated fatty acids (Poster summary), Suppl. 37, 56
Fisher, C. *see* McLelland, J.
Fisher, C. *see* Sharpe, G.R.
Flavell, D.J. *see* Fleming, S.

Fleegler, E.J. *see* Guitart, J.

Fleming, P. *see* Kennedy, C.T.C.

Fleming, R. *see* Rademaker, M.

Fleming, S., Kelly, S.E., McVittie, E., Hunter, J.A.A., Flavell, D.J. and Jones, D.B. Recognition of a stem cell glycoprotein on basal keratinocytes (Paper summary), 818

Fleming, S. *see* Kelly, S.E.

Fletcher, A. *see* Chambers, B.

Fletcher, A. *see* Graham-Brown, R.A.C.

Flower, R.J. *see* Rivers, J.K.

Fluckiger, R. *see* Bircher, A.J.

Foreman, J.C. *see* Bunker, C.B.

Forsyth, A. *see* Docherty, V.R.

Forsyth, A. *see* Garioch, J.

Forsyth, A. *see* Todd, P.

Foulds, I.S. *see* Carmichael, A.J.

Foulds, I.S. *see* Duhra, P.

Francis, D.M. *see* Grattan, C.E.H.

Francis, D.M. *see* Oliver, F.

Frank, M. *see* Cherry, G.M.

Frankel, S.J. *see* Shalom, S.D.

Frenk, E. *see* Merot, Y.

Friedland, J.S. *see* Godfrey, K.M.

Friedmann, P.S., Wren, F., Buffey, J. and MacNeil, S. Alpha-MSH causes a small rise in cAMP but has no effect on basal or ultraviolet-stimulated melanogenesis in human melanocytes, 145

Frith, P., Allen, J. and Wojnarowska, F. Cicatricial and bullous pemphigoid: does the composition of the BMZ of normal skin, oral mucosa and conjunctiva explain the clinical differences? (Paper summary), 817

Frith, P.A. *see* Dalziel, K.L.

Fritsch, D. *see* Hintner, H.

Fry, L. *see* McFadden, J.

Fry, L. *see* Powles, A.V.

Gane, J. *see* Bull, R.H.

Gane, J. *see* Fenton, D.A.

Gane, J.N. *see* Proby, C.M.

Garioch, J., Todd, P., Lamey, P.J., Lewis, M. Forsyth, A. and Rademaker, M. The significance of a positive patch test to mercury in oral disease (Paper summary), Suppl. 37, 25

Garioch, J., Todd, P., Rademaker, M. and Forsyth, A. Allergic contact dermatitis reactions to latex examination gloves (Paper summary), Suppl. 37, 24

Garioch, J. *see* Todd, P.

Garioch, J.J. *see* Rademaker, M.

Garioch, P. *see* Todd, P.

Garrod, D.R. *see* Burge, S.M.

Gawkrodger, D.J. *see* McDonagh, A.J.G.

Gawkrodger, D.J. *see* Sibenge, S.

Gaylarde, P.M. and Sarkany, I. Is 'venous stasis' outmoded? (Correspondence), 273

Gearing, A.J.H. *see* Lawlor, F.

Gearing, A.J.H. *see* Ross, J.S.

Gemell, C. *see* Todd, P.

Gibson, A.G. *see* Williams, R.E.A.

Gibson, B. *see* Kirkham, N.

Gibson, W.T. *see* Taylor, M.

Gibson, W.T. *see* Watson, L.P.

Giddings, J. *see* Mourad, M.M.

Gilkes, J.J.H. *see* Rushton, D.H.

Glover, M. *see* Salisbury, J.A.

Glover, M.T., Lake, B.D. and Atherton, D.J. Infantile systemic hyalinosis: a newly recognised disorder of collagen? (Paper summary), Suppl. 37, 36

Godfrey, K., Wojnarowska, F. and Leonard, J. Linear IgA disease of adults: association with lymphoproliferative malignancy and possible role of other triggering factors, 447

Godfrey, K.M., Wojnarowska, F. and Friedland, J.S. Obliterative bronchiolitis and alveolitis associated with sulphamethoxypyridazine (Lederkyn) therapy for linear IgA disease of adults (Correspondence), 125

Godfrey, K.M. and James, M.P. Treatment of severe acne with isotretinoin in patients with inflammatory bowel disease, 653

Godfrey, K.M. and James, M.P. Benign cephalic histiocytosis: a case report, 245

Goodfield, M.J.D. *see* Harland, C.C.

Goodfield, M.J.D. *see* Sheehan-Dare, R.A.

Goodwin, E.F.J. *see* Safford, R.J.

Goodyear, H.M., Laidler, P.W., Price, E.H., Kenny, A.P. and Harper, J.I. A clinico-microbiological study of toxic erythemas in children (Paper summary), Suppl. 37, 18

Goulding, N.J. *see* Rivers, J.K.

Goutal, H. *see* Machet, L.

Gowers, L. *see* Anstey, A.

Gowland, G. *see* Ashbee, H.R.

Graham-Brown, R.A.C., Osborne, J.E., London, S.P. and Fletcher, A. Public education for melanoma in Leicestershire: an update (Paper summary), Suppl. 37, 38

Grant, A.W., Edward, M. and MacKie, R.M. Basal cell carcinoma derived factor(s) induce fibroblasts to modify the extracellular matrix (Poster summary), 833

Grattan, C.E.H., Francis, D.M. and Greaves, M.W. A histamine releasing factor in serum of chronic urticaria with anti-IgE autoantibody-like properties (Paper summary), Suppl. 37, 45

Greaves, M.W. *see* Grattan, C.E.H.

Greaves, M.W. *see* Handfield-Jones, S.E.

Greaves, M.W. *see* Kobza Black, A.

Greaves, M.W. *see* Lawlor, F.

Greaves, M.W. *see* McGrath, J.A.

Greaves, M.W. *see* Oliver, F.

Greaves, M.W. *see* Rivers, J.K.

Green, A. and Martin, N.G. Measurement and perception of skin colour in a skin cancer survey, 77

Green, M.R. *see* Philpott, M.P.

Griffiths, W.A.D. Nail-blanching: a new cutaneous sign indicating mechanical and functional impairment of the finger pulp (Paper summary), Suppl. 37, 30

Griffiths, C.E.M. *see* Barker, J.N.W.N.

Groves, R.W., MacDonald, L.M. and MacDonald, D.M. Profound digital collagen atrophy: a new cutaneous presentation of adrenal-dependent Cushing's syndrome, 667

Groves, R.W., Allen, M.H., Barker, J.N.W.N., Haskard, D.O. and MacDonald, D.M. Endothelial leukocyte adhesion molecule-1 expression in eczema and psoriasis: a quantitative study (Paper summary), 829
Groves, R.W., Wilson Jones, E. and MacDonald, D.M. Human orf and milkers' nodules: a clinicopathological study (Paper summary), Suppl. 37, 117
Gudmundsson, J. *see* Rademaker, M.
Guitart, J., Bergfeld, W.F., Tuthill, R.J., Tubbs, R.R., Zienowicz, R. and Fleegler, E.J. Squamous cell carcinoma of the nail bed: a clinicopathological study of 12 cases, 215
Gumowski-Sunek, D. *see* Chatellard Gruaz, D.
Gunner, D.B. *see* Eady, R.A.J.
Guzzo, C. and Lazarus, G. Urticaria pigmentosa: systemic evaluation and successful treatment with topical steroids (Paper summary), Suppl. 37, 45

Hagari, Y. *see* Tanaka, K.
Hamada, K. *see* Thornton, M.J.
Hamilton, A.J. *see* McGregor, J.
Handfield-Jones, S.E., Kobza Black, A. and Greaves, M.W. Effects of a bradykinin antagonist, NPC 567 on human skin (Poster summary), 834
Haneke, E. Fluconazole levels in human epidermis and blister fluid (Correspondence), 273
Hannuksela, M. *see* Oikarinen, A.
Harcourt, S.A. *see* Arlett, C.F.
Harland, C.C., Goodfield, M.J.D. and Evans, E.G.V. A follow-up study of terbinafine (SF-327, Lamisil) in the treatment of onychomycosis (Paper summary), Suppl. 37, 31
Harland, C.C., Madeley, R.J. and Millard, L.G. Patient information leaflets in the dermatology out-patient department (Paper summary), Suppl. 37, 35
Harman, R.R.M. *see* Reynolds, N.J.
Harman, R.R.M. *see* Sansom, J.
Harper, J. *see* Judge, M.
Harper, JI, *see* Goodyear, H.M.
Harper, J.I. *see* Judge, M.R.
Harrington, C.I. *see* McDonagh, A.J.G.
Harris, D.W.S., Benton, E.C. and Hunter, J.A.A. The changing face of dermatology out-patient referrals in the south-east of Scotland, 745
Harris, D.W.S., Benton, E.C. and Hunter, J.A.A. Dermatological audit: facts or friction (Paper summary), Suppl. 37, 21
Harris, D.W.S. *see* Colver, G.B.
Harvey, I. *see* Shalom, S.D.
Hashimoto, K., Matsumoto, K., Higashiyama, M., Nishida, Y. and Yoshikawa, K. Growth-inhibitory effects of 1,25-dihydroxyvitamin D_3 on normal and psoriatic keratinocytes, 93
Hashizume, H. *see* Iwatsuki, K.
Haskard, D.O. *see* Groves, R.W.
Haustein, U.F. *see* Herrmann, K.
Haustein, U-F. *see* Rustin, M.H.A.
Hawk, J.L.M. *see* Murphy, G.M.
Hawk, J.L.M. *see* Ross, J.S.
Hay, R.J. *see* McGregor, J.
Hay, R.J. *see* Murdoch, M.E.
Hay, R.J. *see* Robles Martinez, W.
Hayakawa, K. *see* Kikuchi, A.
Hazaz, B. *see* Hodak, E.
Heagerty, A.H.M. *see* Eady, R.A.J.
Heckmann, M. *see* Herrmann, K.
Heeres, K. *see* De Jong, M.C.J.M.
Heggie, R. *see* Edwards, C.
Hein, R. *see* Schirren, C.G.
Henderson, M.J. *see* Sheehan-Dare, R.A.
Herriot, R., Ormerod, A.D. and Sewell, H.F. An immunophenotypic and flow cytometric investigation of adult mastocytosis (Paper summary), Suppl. 37, 57
Herrmann, K., Schulze, E., Heckmann, M., Schubert, I., Meurer, M., Ziegler, V., Haustein, U.F., Mehlhorn, J. and Krieg, T. Type III collagen aminopropetide and laminin P1 levels in serum of patients with silicosis-associated and idiopathic systemic scleroderma, 1
Heule, F., Vuzevski, V.D. and Tio, T.T. Netherton's syndrome (Case report), 269
Higashiyama, M. *see* Hashimoto, K.
Higgins, E.M. *see* Du Vivier, A.W.P.
Higgins, E.M. *see* Farr, P.M.
Hill, S. *see* Marks, R.
Hill, S.E. *see* Burchill, S.A.
Hindson, C., Downey, A., Sinclair, S. and Cominos, B. PUVA therapy of chronic actinic dermatitis: a 5-year follow-up (Correspondence), 273
Hintner, H., Sepp, N., Dahlback, K., Dahlback, B., Fritsch, D. and Breathnach, S. Deposition of C3, C9 neoantigen and vitronectin (S-protein of complement) in lichen planus pemphigoides, 39
Hird, P. *see* Denton, K.J.
Hird, P.M. *see* McDonagh, A.J.G.
Hirohashi, S. *see* Kikuchi, A.
Hodak, E., Kremer, I., David, M., Hazaz, B., Rothem, A., Feuerman, P. and Sandbank, M. Conjunctival involvement in pemphigus vulgaris: a clinical, histopathological and immunofluorescence study, 615
Hodgson, C. *see* Duhra, P.
Holden, C.A. *see* Mallett, R.B.
Holden, C.A. *see* Myint, S.
Holland, K.T. *see* Ashbee, H.R.
Hollema, H. *see* De Jong, M.C.J.M.
Hollema, H. *see* Keukens, F.M.C.
Hollema, H. *see* Meijer, P.
Hollema, H. *see* Van Gerven, C.C.M.
Hollema, H. *see* Van Voorst Vader, P.C.
Holmes, J. *see* Duhra, P.
Honigsmann, H. *see* Petzelbauer, P.
Hoogkamp-Korstanje, J.A.A. *see* Tazelaar, D.J.
Humphreys, F. H1 antagonism reveals the non-histamine component of antigen weals: a model for further study? (Poster summary), Suppl. 37, 58
Hunter, J.A.A. *see* Fleming, S.
Hunter, J.A.A. *see* Harris, D.W.S.
Hunter, J.A.A. *see* Kelly, S.E.
Hutchinson, P.E. *see* Berth-Jones, J.

Iamaizumi, S. *see* Iwatsuki, K.
Ilchyshyn, A. *see* Duhra, P.

ILDERTON, E. *see* CARTWRIGHT, P.H.
INGHAM, E. *see* ASHBEE, H.R.
ISHIDA-YAMAMOTO, A., CASSELLA, J.P., SHIMIZU, H., PURKIS, P.E., LEIGH, I.M. and EADY, R.A.J. Light and electron microscopic localization of different keratins in normal human skin using a 1-nm immunogold silver-enhanced staining method (Paper summary), Suppl. 37, 122
ISHIDA-YAMAMOTO, A., MCGRATH, J.A., CHAPMAN, S.J., LEIGH, I.M. and EADY, R.A.J. Distribution and significance of tonofilament clumping in the Dowling–Meara form of epidermolysis bullosa simplex (DB-EBS) (Poster summary), 834
ITO, S. *see* BURCHILL, S.A.
IWATSUKI, K., IMAIZUMI, S., HASHIZUME, H., SUGATA, K., TAKIGAWA, M. and YAMADA, M. Production of antikeratin autoantibodies by hybrid spleen cells of naive mice, 735

JABŁOŃSKA, S. *see* BLASZCZYK, M.
JACKSON, J. and BARNES, L. Anti-neutrophil cytoplasmic antibodies in dermatitis herpetiformis (Paper summary), Suppl. 37, 14
JAHODA, C.A.B. *see* MICHIE, H.J.
JACOBSEN, B.K. *see* OXHOLM, A.
JAMES, J. *see* RUSTIN, M.H.A.
JAMES, K.C. *see* RUSHTON, D.H.
JAMES, M.P. *see* GODFREY, K.M.
JARZABEK-CHORZELSKA, M. *see* BLASZCZYK, M.
JASSIM, D. *see* DUHRA, P.
JOHNSON, M. and SHUSTER, S. Ventral nail contribution to the nail plate is continuous along the length of the nail bed (Paper summary), 825
JOHNSON, B.E. *see* FERGUSON, J.
JOHNSTON, C.F. *see* EEDY, D.J.
JONES, S.K. and MOSELEY, H. Clear UV lenses: cosmetically acceptable spectacles for PUVA patients (Paper summary), Suppl. 37, 58
JONES, S.K., WALLINGTON, T.B. and KENNEDY, C.T.C. Eczema, thrombocytopenia and recurrent infection: Wiskott–Aldrich syndrome? (Case report), Suppl. 37, 69
JONES, B. *see* MURDOCH, M.E.
JONES, D.B. *see* FLEMING, S.
JONES, S.K. *see* MOSELEY, H.
JONKMAN, M.F., COENEN, J.M.F.H., BRUIN, P. and PENNINGS, A.J. Development of a highly vapour-permeable wound dressing (Paper summary), 67[illegible]
JONKMAN, M.F. *see* DE JONG, M.C.J.M.
JOSEPH, A.E.A. *see* PROBY, C.M.
JOSEPH, A.G.A. *see* BULL, R.H.
JUBIANTO, J. *see* ENGELKENS, H.J.H.
JUDGE, M.R., LAKE, B.D., SMITH, V.V., BESLEY, G.T. and HARPER, J.I. Sjogren–Larrson syndrome: a disorder of fatty alcohol metabolism (Paper summary), Suppl. 37, 16
JUDGE, M.R., MANKU, M.S., STEWART, C. and HARPER, J.I. Analysis of stratum corneum lipids in the hereditary ichthyoses by high performance thin layer chromatography (HPTLC) (Paper summary), 8[illegible]
JUDGE, M.R., MICHAELS, M., SAMS, V.R., DAVID, T.J. and HARPER, J.I. Disseminated porokeratosis in an infant with craniosynostosis, 249
JUDGE, M., HARPER, J. The ichthyoses: a re-appraisal based on a study of 100 patients (Paper summary), Suppl. 37, 50

KALLIOINEN, M. *see* OIKARINEN, A.
KALTER, D.C. *see* ELLNER, K.M.
KANG, S. and DOVER, J.S. Successful treatment of eruptive pyoderma gangrenosum with intravenous vancomycin and mezlocillin, 389
KANO, Y., SHIOHARA, T., YAGITA, A. and NAGASHIMA, M. Granulomatous cheilitis and Crohn's disease, 409
KANWAR, A.J., KAUR, S. Pityriasis rubra pilaris in Indians (Correspondence), 692
KARVONEN, J. *see* OIKARINEN, A.
KATO, T. *see* TERUI, T.
KATZ, D. *see* BRICKELL, P.M.
KAUFMANN, R. *see* EBERHARD KLEIN, C.
KAUR, S. *see* KANWAR, A.J.
KEALEY, T. *see* PHILPOTT, M.P.
KEEFE, M., WHITE, J.E. and PERKINS, P. Nodular melanomas in the over-50 age group: the next target for health education (Paper summary), Suppl. 37, 59
KEIJSER, M.H. Histiocytosis X (Langerhans cell histiocytosis) (Case report), 536
KEIJSER, M.H. *see* STARINK, T.M.
KELLETT, J.K. and EAD, R.D. Treatment of lichen planus with a short course of oral prednisolone (Correspondence), 550
KELLY, S.E., FLEMING, S. and BLACK, M.M. The comparative immunology of the specific dermatoses of pregnancy (Paper summary), Suppl. 37, 59
KELLY, S.E., HUNTER, J.A.A. and FLEMING, S. Calcium dependent localisation of calgranulins in keratinocytes in inflammatory dermatoses (Poster summary), 835
KELLY, S.E. *see* FLEMING, S.
KEMMETT, D. and BARNETSON, R.ST.C. Topical nedocromil sodium: a double-blind placebo-controlled study in atopic dermatitis (Poster summary), Suppl. 37, 60
KENDAL, M.D. *see* FENTON, D.A.
KENNEDY, C.T.C. and DUNCAN, A. Goldenhar syndrome with unilateral trigeminal trophic syndrome (Case report), Suppl. 37, 80
KENNEDY, C.T.C. Lymphangiectasias of the vulva following hysterectomy and radiotherapy (Case report), Suppl. 37, 92
KENNEDY, C.T.C., BURTON, P.A. and COOK, P. Swollen toe due to epithelioid haemangioma of bone (Case report), Suppl. 37, 85
KENNEDY, C.T.C., FLEMING, P. and DUNCAN, A. Eruptive cutaneous and visceral neonatal haemangiomatosis (Case report), Suppl. 37, 83
KENNEDY, C.T.C. *see* JONES, S.K.
KENNEDY, C.T.C. *see* POTTER, M.N.
KENNEDY, C.T.C. *see* REYNOLDS, N.J.
KENNEDY, C.T.C. and WALLINGTON, T. Familial chronic mucocutaneous candidosis with autoimmune polyendocrinopathy, alopecia and resistance to antifungal therapy (Case report), Suppl. 37, 75

Kenny, A.P. *see* Goodyear, H.M.

Keukens, F.M.C., Van Voorst Vader, P.C., De Jong, M.C.J.M. and Hollema, H. Annular elastolytic giant cell granuloma (Case report), 685

Khallouf, R. *see* Machet, L.

Khan, G.K. *see* Finlay, A.Y.

Kikuchi, A., Amagai, M., Hayakawa, K., Ueda, M., Hirohashi, S., Shimizu, N. and Nishikawa, T. Association of EGF receptor expression with proliferating cells and of *ras* p21 expression with differentiating cells in various skin tumours, 49

Kirby, J.D. *see* Mayou, S.C.

Kirkham, N., Gibson, B. and Price, M.L. Breslow thickness of melanoma can be measured in frozen sections (Paper summary), Suppl. 37, 123

Kirtschig, G., Venning, V.A. and Wojnarowska, F. Long term studies in bullous pemphigoid: characterizing the target antigens (Poster summary), 835

Kobza Black, A. and Greaves, MW. Cutaneous reactions database closure (Correspondence), 277

Kobza Black, A. *see* Handfield-Jones, S.E.

Kobza Black, A. *see* Lawlor, F.

Kobza Black, A. *see* Newton, J.A.

Kobza Black, A. *see* Oliver, F.

Kolacinkska-Strasz, A. *see* Blaszczyk, M.

Kolbach, D.N. and Nieboer, C. Fumaric acid therapy in psoriasis: a long-term retrospective study on the effect of fumaric acid combination (FAC-EC) therapy and dimethyl-fumaric acid ester (DMFAE) monotherapy (Paper summary), 534

Korner, C. *see* Steinkraus, V.

Korstanje, M.J. *see* Stoof, T.J.

Kovacs, I.B. *see* Mayou, S.C.

Kremer, I. *see* Hodak, E.

Krieg, T. *see* Schirren, C.G.

Krieg, T. *see* Herrmann, K.

Krunic, A. *see* Stevanovic, D.V.

Kumar, V. *see* Blaszczyk, M.

Kunne, N. *see* Sterry, W.

Kurgansky, D. *see* Beacham, B.E.

Kürkçüoğlu, N. and Alaybeyi, F. Topical capsaicin for psoriasis (Correspondence), 549

Kusuda, S. *see* Yoshida, M.

Laidler, P.W. *see* Goodyear, H.M.

Laing, T. *see* Thornton, M.J.

Lake, B.D. *see* Eady, R.A.J.

Lake, B.D. *see* Glover, M.T.

Lake, B.D. *see* Judge, M.R.

Lamey, P.J. *see* Garioch, J.

Lamey, P.J. *see* Todd, P.

Lane, B. *see* Markey, A.C.

Lane, E.B. *see* Neill, S.M.

Langer, K. *see* Honigsmann, H.

Langer, K. *see* Petzelbauer, P.

Langtry, J.A.A. and Diffey, B.L. The effect of topical solvents on UVB erythema (Paper summary), 821

Lanigan, S.W. and Cotterill, J.A. The treatment of port wine stains with the carbon dioxide laser, 229

Lanigan, S.W. *see* Cotterill, J.A.

Lanternier, G. *see* Machet, L.

Larsson, J. *see* Apelqvist, J.

Latham, J.A.E., Rees, J.L. and Redfern, C.P.F. The expression of retinoic acid receptors in human sebaceus glands (Poster summary), 836

Latham, J. *see* Redfern, C.P.F.

Lauerma, A.I., Rasenen, L., Reunala, T. and Reitamo, S. Langerhans cells but not monocytes are capable of antigen presentation *in vitro* in corticosteroid contact hypersensitivity, 699

Lauharanta, J. *see* Timonen, K.

Lawlor, F., Smith, N.P., Camp, R.D.R., Bacon, K.B., Kobza Black, A., Greaves, M.W. and Gearing, A.J.H. Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides, 297

Lawrence, C.M. Only cartilage needs to be removed in the surgical treatment of chondrodermatitis nodularis (Paper summary), Suppl. 37, 41

Lawrence, C.M. *see* Ramsay, B.

Lawrence, C.M. *see* Speight, E.L.

Lawson, R.S. *see* Edwards, B.D.

Layton, A.M., Sheehan-Dare, R.A. and Cunliffe, W.J. A double-blind placebo-controlled trial of topical PUVA in persistent palmoplantar pustulosis (Paper summary), Suppl. 37, 44

Lazarus, G. *see* Guzzo, C.

Le Bourgeois, M. *see* Bodemar, C.

Leigh, I.M. *see* Burge, S.M.

Leigh, I.M. *see* Chapman, S.J.

Leigh, I.M. *see* De Jong, M.C.J.M.

Leigh, I.M. *see* Ishida-Yamamoto, A.

Leigh, I.M. *see* Mallett, R.B.

Leigh, I.M. *see* Markey, A.C.

Leigh, I.M. *see* Neill, S.M.

Leigh, I.M. *see* Salisbury, J.A.

Leigh, I.M. *see* Schofield, O.M.V.

Leigh, I.M. *see* Venning, V.

Leonard, J. *see* Godfrey, K.

Leonard, J.N. *see* McFadden, J.

Leung, R.S.C., Turnbull, A.J., Taylor, J.A., Russell-Jones, R. and Thompson, R.P.H. Neutrophilic zinc levels in psoriasis and seborrhoeic dermatitis, 319

Levene, G. *see* Whittaker, S.

Lever, L. *see* Finlay, A.Y.

Lever, L.R. and Marks, R. The cell kinetics of seborrhoeic warts and the skin on which they arise (Poster summary), 836

Lever, L.R. *see* Nazmi, N.

Lever, R. *see* Williams, R.E.A.

Lewis, M. *see* Garioch, J.

Lewis, M. *see* Todd, P.

Lichtenberg, B. *see* Sterry, W.

Liden, S., Lidelof, B. and Sparen, P. Is benzoyl peroxide carcinogenic? (Correspondence), 129

Lier, J.G. *see* Van Voorst Vader, P.C.

Lindelof, B., Sigurgeirsson, B., Wahlgren, C.F. and Eklund, G. Chronic urticaria and cancer: an epidemiological study of 1155 patients, 453

Lindelof, B. *see* Liden, S.

Litoux, P. *see* Dreno, B.

Lombardi, C. *see* Empinotti, J.C.

London, S.P. *see* Graham-Brown, R.A.C.

Lord, R. *see* Priestley, G.C.

Lorrette, G. *see* Machet, L.
Lovell, C.R. Important plant allergens in the United Kingdom (Poster summary), Suppl. 37, 61
Lovell, C.R. *see* Finnen, M.J.
Lubach, D., Wawrzyniak-Schulz, A., Neukam, D. and Nissen, S. The extension technique: a new method of demonstrating initial lymph vessels in excised human skin, 179
Lucas, S. *see* Murdoch, M.E.
Lucie, N.P. *see* Williams, R.E.A.
Luscombe, D.K. *see* Finlay, A.Y.

MacDonald, D.M. *see* Barker, J.N.W.N.
MacDonald, D.M. *see* Groves, R.W.
MacDonald, D.M. *see* Markey, A.C.
Macdonald Hull, S. *see* Nutbrown, N.
MacFarlane, A.W. *see* Yu, R.C.H.
Machet, L., Vaillant, L., Goutal, H., Lanternier, G., Khallouf, R., Chagnon, J.L. and Lorrette, G. Kawasaki disease in a young adult with perineal rash (Correspondence), 413
MacKie, R.M., Bufalino, R. and Sutherland, C. The effect of pregnancy on melanoma prognosis (Paper summary), Suppl. 37, 40
MacKie, R.M. *see* Docherty, V.R.
MacKie, R.M. *see* Edward, M.
MacKie, R.M. *see* Grant, A.W.
MacKie, R.M. *see* Williams, R.E.A.
MacKinnon, P. *see* Eady, R.A.J.
MacNeil, S., Dobson, J., Bleehen, S.S. and Buffey, J. MSH increases intracellular calcium in melanoma cells (Paper summary), 828
MacNeil, S. *see* Burchill, S.A.
MacNeil, S. *see* Friedmann, P.S.
Maddison, P.J. *see* Rustin, M.H.A.
Madeley, R.J. *see* Harland, C.C.
Magnenat, P. *see* Merot, Y.
Mahesh, M. *see* Sheehan, M.
Maibach, H.I. *see* Cua, A.B.
Mallett, R.B. *see* Myint, S.
Mallett, R.B., Coulson, I.H., Purkis, P.E., Leigh, I.M. and Holden, C.A. An immuno-histochemical analysis of the changes in the immune infiltrate and keratin expression in psoriasis treated with calcipotriol compared with beta-methasone ointment (Poster summary), 837
Manku, M.S. *see* Judge, M.R.
Markey, A.C., Leigh, I.M., Lane, B. and MacDonald, D.M. Changes in keratin phenotype associated with malignant transformation of keratinocytes (Paper summary), Suppl. 37, 121
Markey, A.C., Pitzalis, C., Allen, M.H. and MacDonald, D.M. CD4$^+$/CD45 RO$^+$ helper inducer lymphocytes in skin—*in vivo* evidence of selective uptake (Paper summary), 829
Marks, R., Hill, S. and Barton, S.P. The effects of an abrasive agent on normal skin and on photoaged skin in comparison with topical tretinoin, 457
Marks, R. *see* Black, D.
Marks, R. *see* Edwards, C.
Marks, R. *see* Lever, L.R.
Marks, R. *see* Mourad, M.M.
Marks, R. *see* Nazmi, M.N.
Marks, R. *see* Samdani, A.J.
Marks, R. *see* Shalom, S.D.
Marks, R. *see* Vijayasingam, S.M.
Marsden, R.A. *see* Curley, R.K.
Martin, N.G. *see* Green, A.
Martins, C.R. *see* Empinotti, J.C.
Maschio, A. *see* Brickell, P.M.
Matthew, J.N.S. *see* Farr, P.M.
Matsumoto, K. *see* Hashimoto, K.
Mattes, J.M. *see* Eberhard Klein, C.
Matthews, C. Acrodermatitis chronic atrophicans (Case report), Suppl. 37, 109
Matthews, C.N.A. *see* Reynolds, N.J.
Mayou, S.C., Kirby, J.D. and Kovacs, I.B. Infusion of iloprost in Raynaud's phenomenon: lack of anti-platelet effect but risk involved (Paper summary), Suppl. 37, 48
Mayou, S.C., Ridler, C. and Kirby, J.D. Haemostatic abnormalities in Sneddon's syndrome (Paper summary), Suppl. 37, 35
Mayou, B.J. *see* Schofield, O.M.V.
McBride, M.E. *see* Ellner, K.M.
McDonagh, A.J.G., Cawood, L. and Messenger, A.G. Expression of extracellular matrix in hair follicle mesenchyme in alopecia areata, 717
McDonagh, A.J.G. and Bleehen, S.S. Lipoid proteinosis: immunohistochemical and ultrastructural studies (Case report), Suppl. 37, 127
McDonagh, A.J.G., Gawkrodger, D.J. and Walker, A.E. Chloracne: report of an outbreak with new clinical observations (Paper summary), Suppl. 37, 21
McDonagh, A.J.G., Slater, D.N., Gawkrodger, D.J., Bird, P.M., Harrington, C.I. and Walker, A.E. Unilesional mycosis fungoides: a review of four cases (Paper summary), Suppl. 37, 123
McDonagh, A.J.G., Wright, A.L., Cotton, D.W.K. and Bleehen, S.S. Malignant melanomas in Sheffield 1971–88 (Paper summary), Suppl. 37, 37
McFadden, J.P., Cerio, R., Orchard, G., Quinn, D.G. and Camp, R.D. Expression of 65 kilodalton heat shock protein immunoreactivity in normal and pathological skin (Poster summary), 837
McFadden, J., Leonard, J.N., Powles, A.V., Valdimarsson, H. and Fry, L. Homology between gluten and human connective tissue proteins (Poster summary), Suppl. 37, 61
McGibbon, D.H. *see* Ross, J.
McGrath, J.A. and Greaves, M.W. Aquagenic pruritus and the myelodysplastic syndrome (Correspondence), 414
McGrath, J.A. *see* Ishida-Yamamoto, A.
McGrath, J.A. *see* Rivers, J.K.
McGregor, J., Hamilton, A.J. and Hay, R.J. Inhibition of human lymphocyte blastogenesis by a factor derived from *Trichophyton rubrum* (Paper summary), 826
McLelland, J. and Shuster S. 'Irritants' increase the contact dermatitis response to allergens (Paper summary), 812

McLELLAND, J., FISHER, C., FARR, P.M. and DIFFEY, B.L. The rate of increase but not the threshold of PUVA erythema response corresponds to plasma psoralen level (Paper summary), 823
McLELLAND, J. *see* QUINN, A.G.
McVITTIE, E. *see* FLEMING, S.
MEHLHORN, J. *see* HERRMANN, K.
MEHLHORN, J. *see* RUSTIN, M.H.A.
MEIJER, P. *see* DE JONG, M.C.J.M.
MEIJER, P., VAN VOORST VADER, P.C., DE JONG, M.C.J.M. and HOLLEMA, H. Porokeratosis disseminata with cutaneous amyloidosis (Case report), 683
MEINARDI, M.M.H.M. *see* DE RIE, M.A.
MELNIK, B.C. and PLEWIG, G. Atopy: a prostaglandin E precursor and receptor dependent defect in T-cell maturation and function? (Correspondence), 126
MENSING, H. *see* STEINKRAUS, V.
MEROT, Y., MIESCHER, P.P., BALSIGER, F., MAGNENAT, P. and FRENK, E. Cutaneous malignant melanomas occurring under cyclosporin A therapy: a report of two cases, 237
MESSENGER, A.G. *see* McDONAGH, A.J.G.
MESSENGER, A.G. *see* RANDALL, V.A.
MESSENGER, A.G. *see* TAYLOR, M.
MESSENGER, A.G. *see* THORNTON, M.J.
MEULENBERG, R., ORANJE, A.P. and VUZEVSKI, V.D. Cutis verticis gyrata (Case report), 260
MEURER, M. *see* HERRMANN, K.
MICHAELS, M. *see* JUDGE, M.R.
MICHIE, H.J., JAHODA, C.A.B., OLIVER, R.F. and POULTON, T.A. Immunobiological studies on the alopecic (DEBR) rat, 557
MIELKE, V. *see* STERRY, W.
MIER, P.D. *see* ARNOLD, W.P.
MIESCHER, P.P. *see* MEROT, Y.
MILLARD, L.G. *see* HARLAND, C.C.
MILLARD, L.G. *see* SHALL, L.
MILLARD, P.R. *see* VENNING, V.
MILLIGAN, A. *see* CHAMBERS, B.
MILPIED, B. *see* DRENO, B.
MITRA, R.S. *see* BARKER, J.N.W.N.
MITRA, R.S. *see* NICKOLOFF, B.J.
MIYAGAWA, S., DOHI, K., YOSHIOKA, A. and SHIRAI, T. Female predominance of immune response to SSA/Ro antigens and risk of neonatal lupus erythematosus, 223
MOLLOY, H. *see* ANSTEY, A.
MOORE, M.K. *see* ROBLES MARTINEZ, W.
MOREL, P. *see* BORRADORI, L.
MORRELL, C.A. *see* ROBLES MARTINEZ, W.
MORRIS, J., DEVEREUX, J., WALKER, S. and CHU, A. Use of human skin in *in vitro* testing of the efficacy of sunblocks in protecting Langerhans cells from UV induced damage (Poster summary), 838
MORRIS, J. *see* DEVEREUX, J.
MORTIMER, P.S. *see* BULL, R.H.
MORTIMER, P.S. *see* FENTON, D.A.
MORTIMER, P.S. *see* PROBY, C.M.
MOSELEY, H. and JONES, S.K. Clear ultraviolet blocking lenses for use by PUVA patients, 775
MOSELEY, H. *see* JONES, S.K.
MOTLEY, R.J. and FINLAY, A.Y. Practical use of a disability index in the routine management of acne (Paper summary), Suppl. 37, 21
MOTT, M.J. *see* POTTER, M.N.
MOURAD, M.M., GIDDINGS, J., MARKS, R., BARTON, S.P. and BAVERSTOCK, J. An immunoradiometric assay for von Willebrand factor antigen to assess angiogenesis, 21
MOWAT, A. *see* DALZIEL, K.L.
MULLER-ESTERL, W. *see* BHOOLA, K.D.
MUNRO, C., REES, J. and SHUSTER, S. Short duration terbinafine therapy penetrates diseased distal nail via the ventral nailbed and is effective in onychomycosis (Paper summary), 825
MURDOCH, M.E., HAY, R.J., RAMNARAIN, N., LUCAS, S., JONES, B. and ABIOSE, A. A clinical classification and grading system of the changes in onchocerciasis and histopathological findings (Paper summary), Suppl. 37, 28
MURPHY, G.M. and HAWK, J.L.M. The incidence of positive antinuclear antibodies and lupus erythematosus in patients presenting with apparent polymorphic light eruption (Paper summary), Suppl. 37, 14
MUSTAJOKI, P. *see* TIMONEN, K.
MYINT, S., MALLETT, R.B. and HOLDEN, C.A. A method for direct measurement of phosphoinositide specific phospholipase C (PLC) activity in mononuclear leucocytes from normal and atopic subjects (Poster summary), 838

NAAFS, B. Micro-organisms, tolerance and autoimmunity (Paper summary), 255
NAGASHIMA, M. *see* KANO, Y.
NAKAGAWA, T. *see* NISHIMOTO, K.
NAKAMURA, K. *see* TAMAKI, K.
NANBA, K. *see* TANAKA, K.
NATER, J.P. *see* TUPKER, R.A.
NATER, J.P. *see* VAN VOORST VADER, P.C.
NAVSARIA, H.A. *see* SCHOFIELD, O.M.V.
NAZMI, M.N., DYKES, P.J. and MARKS, R. Epidermal growth factor receptors in human epidermal tumours, 153
NAZMI, N., LEVER, L.R. and MARKS, R. Effect of isotretinoin on epidermal proliferation (Paper summary), Suppl. 37, 23
NEILL, S.M., ELLIS, J.P., PEMBROKE, A.C. and STAUGHTON, R.C.D. Plantar fibromatosis (Poster summary), Suppl. 37, 62
NEILL, S.M., STAUGHTON, R.C.D., LANE, E.B. and LEIGH, I.M. The cytokeratin profile of normal vulvul epithelium and vulval lichen sclerosus (Poster summary), Suppl. 37, 62
NEUKAM, D. *see* LUBACH, D.
NEWTON, J.A., KOBZA BLACK, A., ARLETT, C.F. and COLE, J. Radiobiological studies in the naevoid basal cell carcinoma syndrome, 573
NICHOLLS, B.M., WORTHY, K., REYNOLDS, N.J., BURTON, J.L. and BHOOLA, K.D. Identification of a plasma kallikrein-like enzyme in human skin blister fluid (Paper summary), 819
NICKOLOFF, B.J. and MITRA, R.S. Direct antiproliferative

effect of cyclosporin A on cultured keratinocytes and fibroblasts (Correspondence), 693
Nickoloff, B.J. *see* Barker, J.N.W.N.
Nicolaides, K.H. *see* Eady, R.A.J.
Nieboer, C. *see* Kolbach, D.N.
Nishida, Y. *see* Hashimoto, K.
Nishikawa, T. *see* Kikuchi, A.
Nishimoto, K., Nishimoto, M., Yamamoto, S., Nakagawa, T., Takaiwa, T. and Tanabe, M. Multiple glomus tumours: successful treatment with electron beam irradiation, 657
Nishimoto, M. *see* Nishimoto, K.
Nissen, S. *see* Lubach, D.
Nolan, N. and Rogers, S. Sclerosing sweat duct carcinoma (microcystic adnexal carcinoma) (Case report), Suppl. 37, 127
Noordhoek, G.T. *see* Engelkens, H.J.H.
Norris, M.J. *see* Rushton, D.H.
Norris, P.G. *see* Ross, J.S.
Nose, M. *see* Steinkraus, V.
Nutbrown, M. *see* Randall, V.A.
Nutbrown, N., Macdonald Hull, S., Thornton, M.J., Cunliffe, W.J. and Randall, V.A. An ultrastructural study of the dermal–epidermal junction in normal and alopecia areata hair follicles (Poster summary), 839

O'Driscoll, J. *see* Edwards, B.D.
Odum, N. *see* Oxholm, A.
Oikarinen, A., Ala-Kokko, L., Tamminen, M., Karvonen, J., Reunala, T., Kallioinen, M. and Hannuksela, M. Effect of long-term PUVA treatment of psoriasis on the collagen and elastin gene expression and growth of skin fibroblasts *in vitro*, 621
Okano, M. and Yoshimoto, K. Commercial assay for steroid sulphatase activity in X-linked ichthyosis (Correspondence), 546
Oliver, F., Amon, E.U., Breathnach, A., Kobza Black, A., Francis, D.M., Sarathchandra, P. and Greaves, M.W. Contact urticaria due to the common stinging nettle (*Urtica dioica*): histological, ultrastructural and pharmacological studies (Poster summary), Suppl. 37, 62
Oliver, A.M. The cytokeratin expression of cultured human foetal keratinocytes, 707
Oliver, R.F. *see* Michie, H.J.
Oranje, A.P. *see* Meulenberg, R.
Oranje, A.P. *see* Van Der Willigen, A.H.
Orchard, G. *see* McFadden, J.P.
Ormerod, A.D. *see* Duncan, J.I.
Ormerod, A.D. *see* Herriot, R.
Ortonne, J.P. *see* Verrando, P.
Osborne, J.E. *see* Graham-Brown, R.A.C.
Oxholm, A., Oxholm, P., Da Cunha Bang, F., Prause, J., Odum, N., Jakobsen, B.K. and Svejgaard, A. Increased frequency of HLA-DR2 in patients with Darier's disease (Correspondence), 845
Oxholm, P. *see* Oxholm, A.

Parker, S.C., Whittaker, S., Smith, N., Spittle, M. and Eady, R.A.J. Adult T-cell leukaemia/lymphoma (Case report), Suppl. 37, 100
Payne, S.N.L. *see* Duncan, J.I.
Peachey, R.D.G. *see* Reynolds, N.J.
Peacock, I. *see* Shall, L.
Pembroke, A.C. *see* Neill, S.M.
Pennings, A.J. *see* Jonkman, M.F.
Perkins, P. *see* Keefe, M.
Perkins, P. *see* White, J.E.
Petri, J. *see* Brandrup, F.
Petzelbauer, P., Honigsmann, H., Langer, K., Anegg, B., Strohal, R., Tanew, A. and Wolff, K. Cyclosporin A in combination with photochemotherapy (PUVA) in the treatment of psoriasis, 641
Philpott, M.P., Westgate, G.E., Green, M.R. and Kealey, T. Human hair growth *in vitro* (Paper summary), 815
Pinnagoda, J. *see* Tupker, R.A.
Pisani, A. *see* Verrando, P.
Pitzalis, C. *see* Markey, A.C.
Plewig, G. *see* Melnik, B.C.
Poblete, M.T. *see* Bhoola, K.D.
Podgorski, M.R. *see* Rivers, J.K.
Polak, J.M. *see* Bunker, C.B.
Pope, F.M. *see* Anstey, A.
Porter, D.I. *see* Duhra, P.
Posma-Ilic, D. *see* Van Voorst Vader, P.C.
Potter, M.N., Kennedy, C.T.C., Mott, M.J., Sainsbury, C. and Berry, P.J. Congential systemic mastocytosis (Case report), Suppl. 37, 70
Poulton, T.A. *see* Michie, H.J.
Powell, R.J. *see* Sowden, J.M.
Powles, A.V., Baker, B.S., Valdimarsson, H. and Fry, L. Four years' experience of cyclosporin for psoriasis (Paper summary), Suppl. 37, 43
Powles, A.V. *see* McFadden, J.
Prause, J. *see* Oxholm, A.
Prens, E.P., Benner, K. and Van Joost, T. The role of T lymphocytes in the pathogenesis of psoriasis (Paper summary), 256
Price, E.H. *see* Goodyear, H.M.
Price, M.L. *see* Kirkham, N.
Priestley, G.C. and Lord, R. Fibroblast–keratinocyte interactions in psoriasis: failure of psoriatic fibroblasts to stimulate keratinocyte proliferation *in vitro*, 467
Priestley, G.C., Lord, R. and Stavropoulos, P. Inhibition of skin fibroblast metabolism by minoxidil (Poster summary), 839
Pritchard, J. *see* Sheehan, M.P.
Proby, C.M., Gane, J.N., Joseph, A.E.A. and Mortimer, P.S. Investigation of the swollen limb with isotope lymphography, 29
Pryce, D.W. *see* Yu, R.C.H.
Puissant, A. *see* Borradori, L.
Purkis, P.E. *see* Ishida-Yamamoto, A.
Purkis, P.E. *see* Mallett, R.B.

Queen, D. *see* Cherry, G.M.
Quinn, A.G., McLelland, J., Essex, T. and Farr, P.M. Measurement of cutaneous inflammation using a scanning laser Doppler velocimeter (Paper summary), 822
Quinn, D.G. *see* McFadden, J.P.

Rademaker, M., Garioch, J.J., Simpson, N.B. and Wallace, M. Isotretinoin treatment alters steroid metabolism in women with acne (Paper summary), 816

Rademaker, M., Simpson, N.B., Gudmundsson, J., Bonduelle, M., Fleming, R. and Coutts, J.R.T. Effect of gonadotrophin releasing hormone analogue (Zoladex) on sebum excretion rates and hair size in hirsute women (Poster summary), 840

Rademaker, M. *see* Garioch, J.

Rademaker, M. *see* Shuttleworth, D.

Rademaker, M. *see* Todd, P.

Ramnarain, N. *see* Murdoch, M.E.

Rampen, F.H.J. *see* Van Everdingen, J.J.

Ramsay, B. and Lawrence, C.M. The manner of plaque clearance during anthralin therapy (Poster summary), 840

Ramsay, B. *see* Speight, E.L.

Ramsay, I.D. *see* Rushton, D.H.

Randall, V.A., Thornton, M.J., Nutbrown, M. and Messenger, A.G. Beard and scalp dermal papilla cells secrete similar mitogenic factors but exhibit different responses to them (Paper summary), 815

Randall, V.A. *see* Nutbrown, N.

Randall, V.A. *see* Thornton, M.J.

Ransom, J.H. *see* Sowden, J.M.

Rasenen, L. *see* Lauerma, A.I.

Redfern, C.P.F., Latham, J., Daly, A. and Todd, C. Retinoic acid receptor expression in 591 melanoma cells (Paper summary), 827

Redfern, C.P.F. *see* Latham, J.A.E.

Redfern, C.P.F. *see* Rees, J.L.

Rees, J. *see* Munro, C.

Rees, J.L. and Redfern, C.P.F. The expression of RAR-alpha, RAR-beta and RAR-gamma in human keratinocytes and dermal fibroblasts *in vitro* (Paper summary), 827

Rees, J.L. *see* Latham, J.A.E.

Reeves, S. *see* Wilkinson, S.M.

Reitamo, S. *see* Lauerma, A.I.

Requena, L. and Yus Sanchez, E. Pigmented spindle cell naevus, 757

Reunala, T. *see* Lauerma, A.I.

Reunala, T. *see* Oikarinen, A.

Reynolds, N.J., Matthews, C.N.A. and Burton, J.L. Strimmer dermatitis (Poster summary), Suppl. 37, 63

Reynolds, N.J., Cobby, M., Bradfield, J.W. and Peachey, R.D.G. Limb hypertrophy associated with a solitary plexiform neurofibroma? (Case report), Suppl. 37, 89

Reynolds, N.J. and Kennedy, C.T.C. Erythema nodosum and cutaneous vasculitis associated with recurrence of Hodgkin's disease (Case report), Suppl. 37, 101

Reynolds, N.J., Rooney, N., Archer, C.B. and Kennedy, C.T.C. Differentiation between pseudolymphoma and malignant B-cell lymphoma of the skin (Case report), Suppl. 37, 95

Reynolds, N.J., Archer, C.B. and Harman, R.R.M. Lymphangiomata following surgery and radiotherapy for breast carcinoma (Case report), Suppl. 37, 93

Reynolds, N.J. and Kennedy, C.T.C. Keratitis ichthyosis deafness (KID) syndrome (Case report), Suppl. 37, 77

Reynolds, N.J. *see* Bhoola, K.D.

Reynolds, N.J. *see* Nicholls, B.M.

Ridler, C. *see* Mayou, S.C.

Rietmayer, C.A.M. *see* Van Der Berg, G.

Rijlaarsdam, J.U. and Willemze, R. Primary cutaneous large cell lymphoma of follicular centre cell origin (Case report), 536

Rijlaarsdam, J.U., Boorsma, D.M., De Haan, P., Sampat, S. and Willemze, R. The significance of Leu 8 negative T cells in lymphoid skin infiltrates: malignant transformation, selective homing or T-cell activation?, 587

Rijlaarsdam, J.U. Angiosarcoma (Case report), 537

Rijlaarsdam, J.U. Smooth muscle hamartoma (Case report), 539

Rivers, J.K., Podgorski, M.R., Goulding, N.J., Wong, E., McGrath, J.A., Flower, R.J. and Greaves, M.W. The presence of autoantibody to recombinant lipocortin-1 in patients with psoriasis and psoriatic arthritis, 569

Rivitti, E.A. *see* Empinotti, J.C.

Robinson, T.W.E. *see* Rustin, M.H.A.

Robles Martinez, W., Bhogal, B., Morrell, C.A., Moore, M.K. and Hay, R.J. The use of fluorescent lectin stains to identify fungi in clinical material from skin (Poster summary), Suppl. 37, 64

Rodeck, C.H. *see* Eady, R.A.J.

Rogers, S. *see* Nolan, N.

Rokugo, M. *see* Terui, T.

Rollman, O. *see* Vahlquist, A.

Rooney, N. *see* Reynolds, N.J.

Ross, J.S., Cerio, R. and Camp, R.D.R. The effect of autologous interleukin 1 on factor XIIIa dermal dendritic cells in normal human skin (Paper summary), Suppl. 37, 117

Ross, J.S., Norris, P.G., Hawk, J.L.M. and Camp, R.D.R. Long-term cyclosporin A in eczematous disorders (Paper summary), Suppl. 37, 50

Ross, J. and McGibbon, D.H. Pseudo-Degos' disease (Case report), Suppl. 37, 111

Ross, J.S., Bacon, K.B., Fincham, N.J., Gearing, A.J.H. and Camp, R.D.R. Mediators of leucocyte infiltration in human skin: a review findings (Poster summary), Suppl. 37, 64

Ross, J.S., Bird, C.R. and Camp, R.D.R. Effect of topical steroid on the recovery of interleukin-1 from normal human skin (Poster summary), 841

Roth, W.K. *see* Schirren, C.G.

Rothem, A. *see* Hodak, E.

Rowell, N.R. *see* Sheehan-Dare, R.A.

Ruiter, D.J. *see* Van Everdingen, J.J.

Rushton, D.H., Ramsay, I.D., James, K.C., Norris, M.J. and Gilkes, J.J.H. Biochemical and trichological characterization of diffuse alopecia in women, 187

Russell-Jones, R. *see* Burrows, N.P.

Russell-Jones, R. *see* Leung, R.S.C.

RUSTIN, M. *see* WHITTAKER, S.

RUSTIN, M.H.A., BULL, H.A., ZIEGLER, V., MEHLHORN, J., HAUSTEIN, U-F. MADDISON, P.J., JAMES, J. and DOWD, P.M. Silica-associated systemic sclerosis is clinically, serologically and immunologically indistinguishable from idiopathic systemic sclerosis, 725

RUSTIN, M.H.A., ROBINSON, T.W.E. and DOWD, P.M. Toxic pustuloderma: a self-limiting eruption, 119

RYAN, J.F. *see* BULL, R.H.

RYAN, T.J. *see* CHERRY, G.M.

RYAN, T.J. *see* DALZIEL, K.L.

RYBOJAD, M. *see* BORRADORI, L.

RYCROFT, R.J.G. *see* ASHWORTH, J.

SAFFORD, R.J., BASKETTER, D.A., ALLENBY, C.F. and GOODWIN, B.F.J. Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol, 595

SAINSBURY, C. *see* POTTER, M.N.

SALEK, M.S. *see* FINLAY, A.Y.

SALISBURY, J.A., GLOVER, M., LEIGH, I.M. and BAKER, H. The PUVA-associated squamous cell carcinoma: a distinct clinico-pathological entity (Poster summary), Suppl. 37, 65

SALOMON, D. and SAURAT, J-H. Erythema multiforme major in a 2-month-old child with human immunodeficiency virus (HIV) infection, 797

SAMDANI, A.J., DYKES, P.J. and MARKS, R. Role of dermatophyte proteinases in the pathogenesis of fungal infection (Paper summary), 824

SAMPAIO, S.A.P. *see* EMPINOTTI, J.C.

SAMPAT, S. *see* RIJLAARSDAM, J.U.

SAMS, V.R. *see* JUDGE, M.R.

SANCHES, J.A. *see* EMPINOTTI, J.C.

SANDBANK, M. *see* HODAK, E.

SANO, Y. *see* TANAKA, K.

SANSOM, J., HARMAN, R.R.M. and COLLINS, C. Infantile digital fibromatosis, diagnosed and treated as neurofibrosarcoma 40 years ago (Case report), Suppl. 37, 92

SARATHCHANDRA, P. *see* OLIVER, F.

SARKANY, I. *see* GAYLARDE, P.M.

SAURAT, J-H. *see* CHATELLARD GRUAZ, D.

SAURAT, J-H. *see* SALOMON, D.

SCHIRREN, C.G., ROTH, W.K., HEIN, R., WERNER, S., KRIEG, T. and BRAUN-FALCO, O. Invasive migration of epidemic Kaposi's sarcoma *in vitro*, 313

SCHOFIELD, O.M.V., CASSELLA, J-P., NAVSARIA, H.A., LEIGH, I.M., MAYOU, B.J. and EADY, R.A.J. Cultured keratinocyte allografts in dystrophic epidermolysis bullosa: preliminary observations (Poster summary), Suppl. 37, 66

SCHOFIELD, O.M.V. *see* EADY, R.A.J.

SCHOFIELD, O.M.V. *see* VERRANDO, P.

SCHUBERT, I. *see* HERRMANN, K.

SCHULZE, E. *see* HERRMANN, K.

SEPP, N. *see* HINTNER, H.

SEWELL, H.F. *see* HERRIOT, R.

SEYWRIGHT, M.M. *see* WILLIAMS, R.E.A.

SHALL, L., MILLARD, L.G., STEVENS, A., TATTERSALL, R.B. and PEACOCK, I. Necrobiosis lipoidica: 'the footprint not the footstep' (Paper summary), Suppl. 37, 47

SHALL, L., STEVENS, A. and MILLARD, L.G. An unusual case of acquired localized argyria, 403

SHALL, L. *see* BARKLEY, A.

SHALOM, S.D., HARVEY, I., MARKS, R. and FRANKEL, S.J. The prevalence of solar keratoses in a South Wales population (Paper summary), Suppl. 37, 40

SHARPE, G.R. and FISHER, C. Time-dependent inhibition of growth of human keratinocytes and fibroblasts by cyclosporin A: effect on keratinocytes at therapeutic blood levels, 207

SHARPE, G.R. and SHUSTER, S. Topical indomethacin aggravates the wealing and flare response in chronic dermographic urticaria (Paper summary), 813

SHARPE, G.R. and SHUSTER, S. Effect of an H1 antagonist (cetirizine) and H2 antagonist (ranitidine) on the symptoms and wealing response in chronic dermographic urticaria (Paper summary), 813

SHARVILL, D.E. *see* ARCHER, C.B.

SHAW, C. *see* EEDY, D.J.

SHAW, C.D. *see* SHUTTLEWORTH, D.

SHEEHAN, M., TSELE, E., MAHESH, M. and CHU, A. Foetal development of epidermal Langerhans cells and T cells (Poster summary), 841

SHEEHAN, M.P., ATHERTON, D.J., BROADBENT, V. and PRITCHARD, J. Topical mechlorethamine: an effective treatment for skin involvement in Langerhans cell histiocytosis (Paper summary), Suppl. 37, 47

SHEEHAN-DARE, R.A., GOODFIELD, M.J.D. and ROWELL, N.R. The influence of indomethacin on disease activity in patients with psoriasis during treatment with the Ingram regime (Paper summary), Suppl. 37, [illegible]

SHEEHAN-DARE, R.A., HENDERSON, M.J. and COTTERILL, J.A. Anxiety and depression in patients with chronic urticaria and generalized pruritus, 769

SHEEHAN-DARE, R.A. *see* LAYTON, A.M.

SHIMAO, S. *see* TANAKA, K.

SHIMIZU, H. *see* ISHIDA-YAMAMOTO, A.

SHIMIZU, N. *see* KIKUCHI, A.

SHIOHARA, T. *see* KANO, Y.

SHIRAI, T. *see* MIYAGAWA, S.

SHUSTER, S. *see* JOHNSON, M.

SHUSTER, S. *see* MCLELLAND, J.

SHUSTER, S. *see* MUNRO, C.

SHUSTER, S. *see* SHARPE, G.R.

SHUTTLEWORTH, D., FINLAY, A.Y., RADEMAKER, M., SHAW, C.D. and SIMPSON, N.B. Psoriasis consultation audit: a two-centre study, 99

SIBENGE, S. and GAWKRODGER, D.J. Rosacea: a survey of presentation patterns and response to therapy (Poster summary), Suppl. 37, 66

SIGURGEIRSSON, B. *see* LINDELOF, B.

SIMPSON, N.B. *see* RADEMAKER, M.

SIMPSON, N.B. *see* SHUTTLEWORTH, D.

SINCLAIR, S. *see* HINDSON, C.

SLATER, D.N. *see* MCDONAGH, A.J.G.

SMITH, D. *see* WILLIAMS, H.C.

SMITH, N. *see* PARKER, S.C.

SMITH, N. *see* WHITTAKER, S.

SMITH, N.P. *see* ARCHER, C.B.

Smith, N.P. *see* Cerio, R.
Smith, N.P. *see* Lawlor, F.
Smith, V.V. *see* Judge, M.R.
Sockalingen, S. *see* Cherry, G.W.
Sonnex, T.S. *see* Cerio, R.
Sowden, J.M., Ransom, J.H., Powell, R.J. and Allen, B.R. Activated peripheral blood T-lymphocytes in atopic dermatitis (Paper summary), Suppl. 37, 49
Sowden, J.M. *see* Brown, R.T.
Sparen, P. *see* Liden, S.
Speight, E.L., Ramsay, B. and Lawrence, C.M. Lack of effect of terfenadine on anthralin inflammation (Paper summary), 812
Spittle, M. *see* Parker, S.C.
Springall, D.R. *see* Bunker, C.B.
Starink, T.M. and Keijser, M.H. Multiple eyelid hidrocystoma, eccrine poromatosis, hypodontia, hypotrichosis syndrome ('Schopf syndrome') (Case report), 543
Starink, T.M. *see* Wikler, J.
Staughton, R.C.D. *see* Bull, R.H.
Staughton, R.C.D. *see* Neill, S.M.
Stavropoulos, P. *see* Priestley, G.C.
Steinkraus, V., Nose, M., Mensing, H. and Korner, C. Radioligand binding characteristics of beta$_2$-adrenoceptors of cultured melanoma cells, 163
Steinmayer, T. *see* Eberhard Klein, C.
Stenstrom, A. *see* Apelqvist, J.
Sterry, W., Bruhn, S., Kunne, N., Lichtenberg, B., Weber-Matthiesen, K., Brasch, J. and Mielke, V. Dominance of memory over naive T cells in contact dermatitis is due to differential tissue immigration, 59
Stevanovic, D.V., Krunic, A. and Djakovic, Z. Pathogenesis in bamboo hair (Paper summary), Suppl. 37, 33
Stevens, A. *see* Shall, L.
Stewart, C. *see* Judge, M.R.
Stewart, T.W. *see* Yu, R.C.H.
Stolz, E. *see* Engelkens, H.J.H.
Stolz, E. *see* Van Ulsen, J.
Stoof, T.J., Korstanje, M.J. and Bilo, H.J.G. Does fish oil protect renal function in cyclosporin-treated psoriasis patients? (Paper summary), 535
Strohal, R. *see* Petzelbauer, P.
Suff, J.S. *see* Delaney, T.J.
Sugaya, K. *see* Iwatsuki, K.
Susskind, W. *see* Todd, P.
Sutherland, C. *see* MacKie, R.M.
Svejgaard, A. *see* Oxholm, A.

Tagami, H. *see* Terui, T.
Takaiwa, T. *see* Nishimoto, K.
Takigawa, M. *see* Iwatsuki, K.
Tamaki, K. and Nakamura, K. Differential enhancement of interferon gamma induced MHC class II expression of HEp-2 cells by 1,25-dihydroxyvitamin D$_3$, 333
Tamminen, M. *see* Oikarinen, A.
Tan, R.S.H. Malignant atrophic papulosis (Degos) (Case report), Suppl. 37, 110
Tanabe, M. *see* Nishimoto, K.
Tanaka, K., Hagari, Y., Sano, Y., Shimao, S. and Nanba, K. A case of T-cell lymphoma associated with panniculitis, progressive pancytopenia and hyperbilirubinemia, 649
Tanew, A. *see* Petzelbauer, P.
Tattersall, R.B. *see* Shall, L.
Taylor, A.E.M. Hereditary woolly hair with ocular involvement, 523
Taylor, J.A. *see* Leung, R.S.C.
Taylor, M., Ashcroft, A.T.T., Messenger, A.G., Westgate, G.E. and Gibson, W.T. Sulphated glycosaminoglycans in the extracellular matrix and medium of cultured human hair papilla and connective tissue sheath cells (Poster summary), 842
Tazelaar, D.J., Velders, A.J., De Koning, J. and Hoogkamp-Korstanje, J.A.A. Acrodermatitis chronica atrophicans (Paper summary), 677
Teillac, D. *see* Bodemar, C.
Tenhunen, R. *see* Timonen, K.
Terenghi, G. *see* Bunker, C.B.
Terui, T., Rokugo, M., Aiba, S., Kato, T. and Tagami, H. Autologous mixed lymphocyte reaction is reduced in patients with psoriasis, 325
Testa, H.J. *see* Edwards, B.D.
Tezuka, T. *see* Yoshida, M.
Thody, A.J. *see* Burchill, S.A.
Thomas, R. *see* Dykes, P.J.
Thomas, R. *see* Finlay, A.Y.
Thompson, D. *see* Whittaker, S.
Thompson, R.P.H. *see* Leung, R.S.C.
Thomson, A.W. *see* Duncan, J.I.
Thomson, J. *see* Todd, P.
Thornton, M.J., Hamada, K., Laing, T., Messenger, A.G. and Randall, V.A. The metabolism of testosterone by cultured dermal papilla cells derived from human male beard follicles (Paper summary), 816
Thornton, M.J. *see* Nutbrown, N.
Thornton, M.J. *see* Randall, V.A.
Tidman, M.J. *see* Chapman, S.J.
Tidman, M.J. *see* Colver, G.B.
Tillman, D.M., White, S.I. and Aitchison, T. Isotretinoin, creatine kinase and exercise (Paper summary), Suppl. 37, 22
Timonen, K., Mustajoki, P., Tenhunen, R. and Lauharanta, J. Effects of haem arginate on variegate porphyria, 381
Tio, T.T., Blindeman, L. and Van Ulsen, J. Pseudoepitheliomatous, keratotic and micaceous balanitis of Lortat-Jacob and Civatte (Case report), 265
Tio, T.T. *see* Heule, F.
Tobin, D.J. *see* Fenton, D.A.
Todd, P., Garioch, J., Lamey, P.J., Lewis, M., Forsyth, A. and Rademaker, M. Patch testing in lichenoid reactions of the mouth and oral lichen planus (Paper summary), Suppl. 37, 26
Todd, C. *see* Redfern, C.P.F.
Todd, P., Garioch, J., Rademaker, M., Susskind, W., Gemell, C. and Thomson, J. Pachyonychia congenita complicated by hidradenitis suppurativa: a family study, 663
Todd, P. *see* Garioch, J.
Tschen, J.A. *see* Ellner, K.M.

Tsele, E. *see* Sheehan, M.
Tubbs, R.R. *see* Guitart, J.
Tupker, R.A., Pinnagoda, J., Coenraads, P.J. and Nater, J.P. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis, 199
Turnbull, A.J. *see* Leung, R.S.C.
Tuthill, R.J. *see* Guitart, J.

Ueda, M. *see* Kikuchi, A.

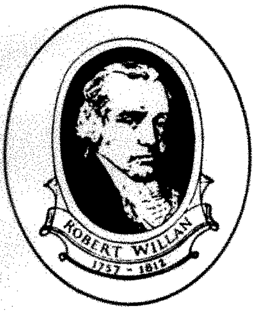
Vahlquist, A. and Rollman, O. Etretinate and the risk for teratogenicity: drug monitoring in a pregnant woman for 9 months after stopping treatment (Correspondence), 131
Vaillant, L. *see* Machet, L.
Valdimarsson, H. *see* McFadden, J.
Valdimarsson, H. *see* Powles, A.V.
Van De Kerkhof, P.C.M. and Weemaes, C.M.R. Skin manifestations in congenital deficiency of leucocyte-adherence glycoproteins (CDLG), 395
Van De Kerkhof, P.C.M. *see* Arnold, W.P.
Van De Kerkhof, P.C.M. *see* De Jong, E.G.J.M.
Van Der Akker, T.W., Van Der Willigen, A.H., Van Der Kwast, T.H. and Van Dongen, J.J.M. Cutaneous T-cell lymphoma after successful treatment of follicular B-cell lymphoma (Case report), 266
Van Der Berg, G., Rietmayer, C.A.M. and Van Joost, T. Dermatitis herpetiformis (Case report), 262
Van Der Kwast, T.H. *see* Van Der Akker, T.W.
Van Der Sluis, J.J. *see* Engelkens, H.J.H.
Van Der Willigen, A.H. and Oranje, A.P. Diffuse cutaneous mastocytosis (Case report), 267
Van Der Willigen, A.H. *see* Van Der Akker, T.W.
Van Dongen, J.J.M. *see* Van Der Akker, T.W.
Van Embden, J. *see* Engelkens, H.J.H.
Van Everdingen, J.J., Rampen, F.H.J., Reiter, D.J. and Casparie, A.F. Evaluation of consensus development conference on cutaneous melanoma in The Netherlands (Paper summary), 259
Van Gerven, C.C.M., Van Voorst Vader, P.C., De Jong, M.C.J.M. and Hollema, H. Anetoderma of Jadassohn–Pellizari (Case report), 686
Van Hooijdonk, C.A.E.M. *see* Arnold, W.P.
Van Joost, T. *see* Prens, E.P.
Van Joost, T. *see* Van Der Berg, G.
Van Luijk, W.H.J. *see* De Jong, M.C.J.M.
Van Ulsen, J., Buitenwerf, J., Dumas, A.M. and Stolz, E. Analysis of the type-specific serological response in herpes simplex virus infections (Paper summary), 258
Van Ulsen, J. *see* Tio, T.T.
Van Voorst Vader, P.C., Coenraads, P.J., Nater, J.P., Lier, J.G. and Woest, T.E. Atopic dermatitis: methodology of patch tests with house dust mite allergens (Paper summary), 675
Van Voorst Vader, P.C., Posma-Ilic, D., De Jong, M.C.J.M. and Hollema, H. Cutis laxa acquisita: the inflammatory variant (Case report), 688
Van Voorst Vader, P.C. *see* De Jong, M.C.J.M.
Van Voorst Vader, P.C. *see* Keukens, F.M.C.
Van Voorst Vader, P.C. *see* Meijer, P.
Van Voorst Vader, P.C. *see* Van Gerven, C.C.M.
Variend, S. *see* Bittiner, S.B.
Velders, A.J. *see* Tazelaar, D.J.
Venning, V., Burge, S.M., Dalziel, K. and Millard, P.R. Pemphigus vegetans masquerading as multiple squamous cell carcinomata (Case report), Suppl. 37, 112
Venning, V., Whitehead, P.H., Leigh, I.M., Allen, J. and Wojnarowska, F. Clinical variants of bullous pemphigoid are not determined by the specificity of the target antigen defined by immunoblotting (Paper summary), Suppl. 37, 15
Venning, V.A. *see* Kirtschig, G.
Venning, V.A. and Wojnarowska, F. The association of bullous pemphigoid and malignant disease: a case control study, 439
Verola, O. *see* Borradori, L.
Verrando, P., Schofield, O.M.V., Pisani, A., Eady, R.A.J. and Ortonne, J.P. Conservation of a subunit of BM-600 (GB3 antigen) in Herlitz syndrome—implications for understanding pathogenesis (Paper summary), 818
Vestey, J.P., Yirrell, D.L. and Aldridge, R.D. A case of human catpox/cowpox infection (Poster summary), Suppl. 37, 67
Vijayasingam, S.M., Dykes, P.J. and Marks, R. Phenytoin has little effect on models of wound healing *in vitro* (Paper summary), Suppl. 37, 17
Vuzevski, V.D. *see* Engelkens, H.J.H.
Vuzevski, V.D. *see* Heule, F.
Vuzevski, V.D. *see* Meulenberg, R.

Wachsman, M., Aurelian, L. and Burnett, J.W. The prophylactic use of cyclooxygenase inhibitors in recurrent herpes simplex infections, 375
Wahlgren, C.F. *see* Lindelof, B.
Walker, A.E. *see* McDonagh, A.J.G.
Walker, S. *see* Devereux, J.
Walker, S. *see* Morris, J.
Wallace, M. *see* Rademaker, M.
Wallington, T. *see* Kennedy, C.T.C.
Wallington, T.B. *see* Jones, S.K.
Watson, L.P., Westgate, G.E., Blount, M.A. and Gibson, W.T. Characterisation of glycosaminoglycans in isolated hair follicles (Poster summary), 843
Wawrzyniak-Schulz, A. *see* Lubach, D.
Weber, L. *see* Eberhard Klein, C.
Weber-Matthiesen, K. *see* Sterry, W.
Wechsler, B. *see* Bodemar, C.
Weemaes, C.M.R. *see* Van De Kerkhof, P.C.M.
Werner, S. *see* Schirren, C.E.
Westeen, B. *see* Finlay, A.Y.
Westgate, G.E. *see* Philpott, M.P.
Westgate, G.E. *see* Taylor, M.
Westgate, G.E. *see* Watson, L.P.
White, I.R. *see* Ashworth, J.
White, J.E., Perkins, P. and Evans, E.G.V. Successful treatment of chronic tinea pedis and tinea manuum with Lamisil (Paper summary), Suppl. 37, 30
White, J.E. *see* Keefe, M.
White, S.I. *see* Tillman, D.M.
Whitehead, P.H. *see* Venning, V.

WHITTAKER, S., RUSTIN, M., LEVENE, G., THOMPSON, D. and SMITH, N. HTLV-1 associated cutaneous disease (Paper summary), Suppl. 37, 29
WHITTAKER, S. *see* PARKER, S.C.
WIKLER, J. Infantile myofibromatosis (multicentric type) (Case report), 540
WIKLER, J. and STARINK, T.M. Acanthosis nigricans-like epidermal naevus and Klippel–Trenaunay syndrome (Case report), 539
WILHELM, K-P. *see* CUA, A.B.
WILKINSON, S.M., CARTWRIGHT, P.H., ALLEN, C., REEVES, S., ALEXANDER, L. and BYRNE, J.P.H. Necrolytic migratory erythema: association with neuroendocrine tumour with predominant insulin secretion, 801
WILKINSON, S.M. *see* CARTWRIGHT, P.H.
WILLEMZE, R. *see* RIJLAARSDAM, J.U.
WILLIAMS, H.C., SMITH, D. and DU VIVIER, A.W.P. Evaluation of public education campaigns in cutaneous melanoma: the King's College Hospital experience, 85
WILLIAMS, H.C. *see* DU VIVIER, A.W.P.
WILLIAMS, R.E.A., GIBSON, A.G., AITCHISON, T.C., LEVER, R. and MACKIE, R.M. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis, 493
WILLIAMS, R.E.A., SEYWRIGHT, M.M., LEVER, R. and LUCIE, N.P. Angiotropic B-cell lymphoma (malignant angioendotheliomatosis): failure of systemic chemotherapy, 807
WILSON, C.L., BURGE, S.M., DEAN, D., DAWBER, R.P.R. and WOJNAROWSKA, F. A clinicopathological study of scarring alopecia in chronic cutaneous lupus erythematosus (Paper summary), Suppl. 37, 13
WILSON JONES, E. *see* CERIO, R.
WILSON JONES, E. *see* GROVES, R.W.
WINFIELD, A.J. *see* DUNCAN, J.I.
WINTER, M. *see* ANSTEY, A.
WOEST, T.E. *see* VAN VOORST VADER, P.C.
WOJNAROWSKA, F. *see* DE BERKER, D.
WOJNAROWSKA, F. *see* FRITH, P.
WOJNAROWSKA, F. *see* GODFREY, K.
WOJNAROWSKA, F. *see* KIRTSCHIG, G.
WOJNAROWSKA, F. *see* VENNING, V.
WOJNAROWSKA, F. *see* WILSON, C.L.
WOLF, J.E. JR. *see* ELLNER, K.M.
WOLFF, K. *see* PETZELBAUER, P.
WONG, E. *see* RIVERS, J.K.
WORTHY, K. *see* NICHOLLS, B.M.
WREN, F. *see* FRIEDMANN, P.S.
WRIGHT, A. *see* DENTON, K.J.
WRIGHT, A.L. *see* MCDONAGH, A.J.G.

YAGITA, A. *see* KANO, Y.
YAMADA, M. *see* IWATSUKI, K.
YAMAMOTO, S. *see* NISHIMOTO, K.
YARDLEY, H.J. *see* CARTWRIGHT, P.H.
YIRRELL, D.L. *see* VESTEY, J.P.
YOSHIDA, M., KUSUDA, S. and TEZUKA, T. Varicella bullosa in an adult (Correspondence), 846
YOSHIKAWA, K. *see* HASHIMOTO, K.
YOSHIMOTO, K. *see* OKANO, M.
YOSHIOKA, A. *see* MIYAGAWA, S.
YU, R.C.H., PRYCE, D.W., MACFARLANE, A.W. and STEWART, T.W. A histopathological study of 643 cutaneous horns (Paper summary), Suppl. 37, 46
YUS SANCHEZ, E. *see* REQUENA, L.

ZIEGLER, V. *see* HERRMANN, K.
ZIEGLER, V. *see* RUSTIN, M.H.A.
ZIENOWICZ, R. *see* GUITART, J.
ZWIERSTRA, R.P. *see* DE JONG, M.C.J.M.



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Contents

No. 1: JULY 1990

Type III collagen aminopropeptide and laminin P1 levels in serum of patients with silicosis-associated and idiopathic systemic scleroderma 1
K.HERRMANN, E.SCHULZE, M.HECKMANN, I.SCHUBERT, M.MEURER, V.ZIEGLER, U.F.HAUSTEIN, J.MEHLHORN AND TH.KRIEG

Ciprofloxacin-induced photosensitivity: *in vitro* and *in vivo* studies 9
J.FERGUSON AND B.E.JOHNSON

An immunoradiometric assay for von Willebrand factor antigen to assess angiogenesis 21
M.M.MOURAD, J.GIDDINGS, R.MARKS, S.P.BARTON AND J.BAVERSTOCK

Investigation of the swollen limb with isotope lymphography 29
C.M.PROBY, J.N.GANE, A.E.A.JOSEPH AND P.S.MORTIMER

Deposition of C3,C9 neoantigen and vitronectin (S-protein of complement) in lichen planus pemphigoides 39
H.HINTNER, N.SEPP, K.DAHLBÄCK, B.DAHLBÄCK, D.FRITSCH AND S.M.BREATHNACH

Association of EGF receptor expression with proliferating cells and of *ras* p21 expression with differentiating cells in various skin tumours 49
A.KIKUCHI, M.AMAGAI, K.HAYAKAWA, M.UEDA, S.HIROHASHI, N.SHIMIZU AND T.NISHIKAWA

Dominance of memory over naive T cells in contact dermatitis is due to differential tissue immigration 59
W.STERRY, SILKE BRUHN, N.KÜNNE, BEATE LICHTENBERG, K.WEBER-MATTHIESEN, J.BRASCH AND V.MIELKE

Vasoactive intestinal peptide (VIP) and peptide histidine methionine (PHM) in human eccrine sweat glands: demonstration of innervation, specific binding sites and presence in secretions 65
D.J.EEDY, C.SHAW, E.P.ARMSTRONG, C.F.JOHNSTON AND K.D.BUCHANAN

Measurement and perception of skin colour in a skin cancer survey 77
ADÈLE GREEN AND N.G.MARTIN

Evaluation of public education campaigns in cutaneous melanoma: the King's College Hospital experience 85
H.C.WILLIAMS, D.SMITH AND A.W.P.DU VIVIER

Growth-inhibitory effects of 1,25-dihydroxyvitamin D_3 on normal and psoriatic keratinocytes 93
K.HASHIMOTO, K.MATSUMOTO, M.HIGASHIYAMA, Y.NISHIDA AND K.YOSHIKAWA

Psoriasis consultation audit: a two-centre study 99
D.SHUTTLEWORTH, A.Y.FINLAY, M.RADEMAKER, C.D.SHAW AND N.B.SIMPSON

Subcutaneous xanthomatosis 107
C.B.ARCHER, D.E.SHARVILL AND N.P.SMITH

Urticarial vasculitis associated with a monoclonal IgM gammopathy: Schnitzler's syndrome 113
L.BORRADORI, M.RYBOJAD, A.PUISSANT, A.DALLOT, O.VEROLA AND P.MOREL

Toxic pustuloderma: a self-limiting eruption 119
M.H.A.RUSTIN, T.W.E.ROBINSON AND PAULINE M.DOWD

Correspondence

Obliterative bronchiolitis and alveolitis associated with sulphamethoxypyridazine (Lederkyn) therapy for linear IgA disease of adults 125
K.M.GODFREY, F.WOJNAROWSKA AND J.S.FRIEDLAND

Atopy: a prostaglandin E precursor- and receptor-dependent defect in T-cell maturation and function? 126
B.C.MELNIK AND G.PLEWIG

Skin diseases that may mimic child abuse 129
G.B.COLVER, D.W.S.HARRIS AND M.J.TIDMAN

Is benzoyl peroxide carcinogenic? 129
S.LIDÉN, B.LINDELÖF AND P.SPARÉN

Etretinate and the risk for teratogenicity: drug monitoring in a pregnant woman for 9 months after stopping treatment 131
A.VAHLQUIST AND O.ROLLMAN

Book Reviews 132

News and Notices 134

No. 2: AUGUST 1990

Abnormal expression of hemidesmosome-like structures by junctional epidermolysis bullosa keratinocytes *in vitro* 137
S.J.CHAPMAN, IRENE M.LEIGH, M.J.TIDMAN AND R.A.J.EADY

α-MSH causes a small rise in cAMP but has no effect on basal or ultraviolet-stimulated melanogenesis in human melanocytes 145
P.S.FRIEDMANN, F.WREN, J.BUFFEY AND S.MACNEIL

Epidermal growth factor receptors in human epidermal tumours 153
M.N.NAZMI, P.J.DYKES AND R.MARKS

Radioligand binding characteristics of β_2-adrenoceptors of cultured melanoma cells 163
V.STEINKRAUS, MONIKA NOSE, H.MENSING AND CHRISTA KÖRNER

Integrins of normal human epidermis: differential expression, synthesis and molecular structure 171
C.EBERHARD KLEIN, T.STEINMAYER, J.M.MATTES, R.KAUFMANN AND L.WEBER

The extension technique: a new method of demonstrating initial lymph vessels in excised human skin 179
D.LUBACH, A.WAWRZYNIAK-SCHULZ, D.NEUKAM AND S.NISSEN

Biochemical and trichological characterization of diffuse alopecia in women 187
D.H.RUSHTON, I.D.RAMSAY, K.C.JAMES, M.J.NORRIS AND J.J.H.GILKES

Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis 199
R.A.TUPKER, J.PINNAGODA, P.J.COENRAADS AND J.P.NATER

Time-dependent inhibition of growth of human keratinocytes and fibroblasts by cyclosporin A: effect on keratinocytes at therapeutic blood levels 207
G.R.SHARPE AND C.FISHER

Squamous cell carcinoma of the nail bed: a clinicopathological study of 12 cases 215
JOAN GUITART, WILMA F.BERGFELD, R.J.TUTHILL, R.R.TUBBS, R.ZIENOWICZ AND E.J.FLEEGLER

Female predominance of immune response to SSA/Ro antigens and risk of neonatal lupus erythematosus 223
S.MIYAGAWA, K.DOHI, A.YOSHIOKA AND T.SHIRAI

The treatment of port wine stains with the carbon dioxide laser 229
S.W.LANIGAN AND J.A.COTTERILL

Cutaneous malignant melanomas occurring under cyclosporin A therapy: a report of two cases 237
Y.MÉROT, P.P.MIESCHER, F.BALSIGER, P.MAGNENAT AND E.FRENK

Discoid lupus erythematosus associated with hereditary angioneurotic oedema 241
P.DUHRA, J.HOLMES AND D.I.PORTER

Benign cephalic histiocytosis: a case report 245
K.M.GODFREY AND M.P.JAMES

Disseminated porokeratosis in an infant with craniosynostosis 249
M.R.JUDGE, M.MICHAELS, V.R.SAMS, T.J.DAVID AND J.I.HARPER

Society Proceedings

Proceedings of the 246th Meeting of the Netherlands Society for Dermatology and Venereology 255

Obituary

D.A.Birkett 271

Correspondence

PUVA therapy of chronic actinic dermatitis: a 5-year follow-up 273
C.HINDSON, ANN DOWNEY, SUSAN SINCLAIR AND BELLE EDMONDS

Fluconazole levels in human epidermis and blister fluid 273
E.HANEKE

Is 'venous stasis' outmoded? 274
P.M.GAYLARDE AND I.SARKANY

Reply 276
J.L.BURTON

Cutaneous reactions database closure 277
ANNE KOBZA BLACK AND M.W.GREAVES

Book Reviews 278

News and Notices 281

No. 3: SEPTEMBER 1990

Effect of topical retinoic acid on the interleukin 1α and β immunoreactive pool in normal human epidermis 283
D.CHATELLARD GRUAZ, L.DIDIERJEAN, D.GUMOWSKI-SUNEK AND J-H.SAURAT

DNA content and $K_s8.12$ binding of the psoriatic lesion during treatment with the vitamin D_3 analogue MC903 and betamethasone 291
S.DE MARE, E.G.J.M.DE JONG AND P.C.M.VAN DE KERKHOF

Skin exudate levels of interleukin 6, interleukin 1 and other cytokines in mycosis fungoides 297
FRANCES LAWLOR, N.P.SMITH, R.D.R.CAMP, K.B.BACON, ANNE KOBZA BLACK, M.W.GREAVES AND A.J.H.GEARING

Elastic fibre damage induced by low-dose D-penicillamine 305
K.L.DALZIEL, S.M.BURGE, P.A.FRITH, T.J.RYAN AND A.MOWAT

Invasive migration of epidemic Kaposi's sarcoma cells *in vitro* 313
C.G.SCHIRREN, W.K.ROTH, R.HEIN, S.WERNER, T.KRIEG AND O.BRAUN-FALCO

Neutrophil zinc levels in psoriasis and seborrhoeic dermatitis 319
R.S.C.LEUNG, A.J.TURNBULL, J.A.TAYLOR, R.RUSSELL-JONES AND R.P.H.THOMPSON

Autologous mixed lymphocyte reaction is reduced in patients with psoriasis 325
T.TERUI, M.ROKUGO, S.AIBA, T.KATO AND H.TAGAMI

Differential enhancement of interferon-γ-induced MHC class II expression of HEp-2 cells by 1,25-dihydroxyvitamin D_3 333
K.TAMAKI AND K.NAKAMURA

Pentoxifylline inhibits the proliferation of human fibroblasts derived from keloid, scleroderma and morphoea skin and their production of collagen, glycosaminoglycans and fibronectin 339
B.BERMAN AND M.R.DUNCAN

Analysis of side-effects of medium-and low-dose cyclosporin maintenance therapy in psoriasis 347
M.A.DE RIE, M.M.H.M.MEINARDI AND J.D.BOS

White piedra: evidence for a synergistic infection 355
K.M.ELLNER, MOLLIE E.MCBRIDE, D.C.KALTER, J.A.TSCHEN AND J.E.WOLF, JR

Ultrasound velocity in human fingernail and effects of hydration: validation of *in vivo* nail thickness measurement techniques 365
A.Y.FINLAY, B.WESTERN AND C.EDWARDS

The prophylactic use of cyclooxygenase inhibitors in recurrent herpes simplex infections 375
M.WACHSMAN, L.AURELIAN AND J.W.BURNETT

Effects of haem arginate on variegate porphyria 381
K.TIMONEN, P.MUSTAJOKI, R.TENHUNEN AND J.LAUHARANTA

Successful treatment of eruptive pyoderma gangrenosum with intravenous vancomycin and mezlocillin 389
S.KANG AND J.S.DOVER

Skin manifestations in congenital deficiency of leucocyte-adherence glycoproteins (CDLG) 395
P.C.M.VAN DE KERKHOF AND C.M.R.WEEMAES

An unusual case of acquired localized argyria 403
L.SHALL, A.STEVENS AND L.G.MILLARD

Granulomatous cheilitis and Crohn's disease 409
Y.KANO, T.SHIOHARA, A.YAGITA AND M.NAGASHIMA

Correspondence

Kawasaki disease in a young adult with a perineal rash 413
L.MACHET, L.VAILLANT, H.GOUTAL, G.LANTERNIER, R.KHALLOUF, J.L.CHAGNON AND G.LORRETTE

Aquagenic pruritus and the myelodysplastic syndrome 414
J.A.MCGRATH AND M.W.GREAVES

Persistent vitiligo induced by diphencyprone 415
P.DUHRA AND I.S.FOULDS

Book Reviews 417

News and Notices 419

No. 4: OCTOBER 1990

Autoantibodies to nucleolar antigens in systemic scleroderma: clinical correlations ... 421
MARIA BŁASZCZYK, MARIA JARZABEK-CHORZELSKA, STEFANIA JABŁOŃSKA, T.CHORZELSKI, ZOFIA KOŁACIŃSKA-STRASZ, E.H.BEUTNER AND V.KUMAR

Endemic pemphigus foliaceus in western Parana, Brazil (1976–1988) ... 431
J.C.EMPINOTTI, L.A.DIAZ, C.R.MARTINS, E.A.RIVITTI, S.A.P.SAMPAIO, C.LOMBARDI, J.A.SANCHES AND COOPERATIVE GROUP FOR FOGO SELVAGEM RESEARCH

The association of bullous pemphigoid and malignant disease: a case control study ... 439
V.A.VENNING AND F.WOJNAROWSKA

Linear IgA disease of adults: association with lymphoproliferative malignancy and possible role of other triggering factors ... 447
K.GODFREY, FENELLA WOJNAROWSKA AND J.LEONARD

Chronic urticaria and cancer: an epidemiological study of 1155 patients ... 453
B.LINDELÖF, B.SIGURGEIRSSON, C.F.WAHLGREN AND G.EKLUND

The effects of an abrasive agent on normal skin and on photoaged skin in comparison with topical tretinoin ... 457
R.MARKS, S.HILL AND S.P.BARTON

Fibroblast–keratinocyte interactions in psoriasis: failure of psoriatic fibroblasts to stimulate keratinocyte proliferation *in vitro* ... 467
G.C.PRIESTLEY AND R.LORD

Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss ... 473
ANASTASIA B.CUA, K-P.WILHELM AND H.I.MAIBACH

Determination of terbinafine in nail samples during systemic treatment for onychomycoses ... 481
P.J.DYKES, R.THOMAS AND A.Y.FINLAY

Epidermal interleukin 1 in normal skin of patients with HIV infection ... 487
B.DRENO, B.MILPIED, H.DUTARTRE AND P.LITOUX

Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis ... 493
R.E.A.WILLIAMS, A.G.GIBSON, T.C.AITCHISON, R.LEVER AND R.M.MACKIE

Effect of incisional biopsy on subsequent histology of melanocytic naevi ... 503
R.K.CURLEY, M.E.FALLOWFIELD, M.G.COOK AND R.A.MARSDEN

Eczematous infiltrated plaques to subcutaneous heparin: a type IV allergic reaction ... 507
A.J.BIRCHER, R.FLÜCKIGER AND S.A.BUCHNER

Regressing atypical histiocytosis and lymphomatoid papulosis: variants of the same disorder? ... 515
R.CERIO AND M.M.BLACK

Hereditary woolly hair with ocular involvement ... 523
AILEEN E.M.TAYLOR

Squamous cell carcinoma complicating hidradenitis suppurativa ... 527
A.V.ANSTEY, J.D.WILKINSON AND P.LORD

Society Proceedings

Proceedings of the 247th Meeting of the Netherlands Society for Dermatology and Venereology ... 533

Correspondence

Efficacy of intravenous immunoglobulins in sclerodermatomyositis ... 546
C.BODEMER, D.TEILLAC, M.LE BOURGEOIS, B.WECHSLER AND Y.DE PROST

Commercial assay for steroid sulphatase activity in X-linked ichthyosis ... 547
M.OKANO AND K.YOSHIMOTO

Sunbed pseudoporphyria induced by nalidixic acid ... 548
D.BILSLAND AND W.S.DOUGLAS

Erosive pustular dermatosis of the leg—a definition ... 549
J.A.COTTERILL AND S.W.LANIGAN

Expression of the myelomonocytic antigens L1 and CD36 in human epidermis ... 549
J.N.W.N.BARKER, M.H.ALLEN, C.E.M.GRIFFITHS, B.J.NICKOLOFF AND D.M.MACDONALD

Topical capsaicin for psoriasis ... 550
N.KÜRKÇÜOĞLU AND F.ALAYBEYI

Treatment of lichen planus with a short course of oral prednisolone ... 551
J.K.KELLETT AND R.D.EAD

Book Reviews ... 553

News and Notices ... 555

No. 5: NOVEMBER 1990

Immunobiological studies on the alopecic (DEBR) rat 557
H.J.MICHIE, C.A.B.JAHODA, R.F.OLIVER AND T.A.POULTON
The presence of autoantibody to recombinant lipocortin-1 in patients with psoriasis and psoriatic arthritis 569
J.K.RIVERS, M.R.PODGORSKI, N.J.GOULDING, E.WONG, J.A.MCGRATH, R.J.FLOWER AND M.W.GREAVES
Radiobiological studies in the naevoid basal cell carcinoma syndrome 573
JULIA A.NEWTON, ANNE KOBZA BLACK, C.F.ARLETT AND JANE COLE
Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin 581
W.P.ARNOLD, CANDIDA A.E.M.VAN HOOIJDONK, P.D.MIER AND P.C.M.VAN DE KERKHOF
The significance of Leu 8 negative T cells in lymphoid skin infiltrates: malignant transformation, selective homing or T-cell activation? 587
J.U.RIJLAARSDAM, D.M.BOORSMA, P.DE HAAN, S.SAMPAT AND R.WILLEMZE
Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol 595
R.J.SAFFORD, D.A.BASKETTER, C.F.ALLENBY AND B.F.J.GOODWIN
Cutaneous sodium lauryl sulphate irritation potential: age and regional variability 607
ANASTASIA B.CUA, K.-P.WILHELM AND H.I.MAIBACH
Conjunctival involvement in pemphigus vulgaris: a clinical, histopathological and immunofluorescence study 615
EMMILIA HODAK, I.KREMER, M.DAVID, B.HAZAZ, A.ROTHEM, P.FEUERMAN AND MIRIAM SANDBANK
Effect of long-term PUVA treatment of psoriasis on the collagen and elastin gene expression and growth of skin fibroblasts *in vitro* 621
A.OIKARINEN, L.ALA-KOKKO, M.TAMMINEN, J.KARVONEN, T.REUNALA, M.KALLIOINEN AND M.HANNUKSELA
Enhanced percutaneous absorption of a novel topical cyclosporin A formulation and assessment of its immunosuppressive activity 631
J.I.DUNCAN, S.N.L.PAYNE, A.J.WINFIELD, A.D.ORMEROD AND A.W.THOMSON
Cyclosporin A in combination with photochemotherapy (PUVA) in the treatment of psoriasis 641
P.PETZELBAUER, H.HÖNIGSMANN, K.LANGER, BARBARA ANEGG, R.STROHAL, A.TANEW AND K.WOLFF
A case of T-cell lymphoma associated with panniculitis, progressive pancytopenia and hyperbilirubinemia 649
K.TANAKA, Y.HAGARI, Y.SANO, S.SHIMAO AND K.NANBA
Treatment of severe acne with isotretinoin in patients with inflammatory bowel disease 653
K.M.GODFREY AND M.P.JAMES
Multiple glomus tumours: successful treatment with electron beam irradiation 657
K.NISHIMOTO, M.NISHIMOTO, S.YAMAMOTO, T.NAKAGAWA, T.TAKAIWA AND M.TANABE
Pachyonychia congenita complicated by hidradenitis suppurativa: a family study 663
P.TODD, J.GARIOCH, M.RADEMAKER, W.SUSSKIND, C.GEMELL AND J.THOMSON
Profound digital collagen atrophy: a new cutaneous presentation of adrenal-dependent Cushing's syndrome 667
R.W.GROVES, L.M.MACDONALD AND D.M.MACDONALD

Society Proceedings

Proceedings of the 248th Meeting of the Netherlands Society for Dermatology and Venereology 673

Correspondence

Acantholytic lesions in the vagina 691
F.BRANDRUP, J.PETRI AND J.AEGIDIUS
Pityriasis rubra pilaris in Indians 692
A.J.KANWAR, SURRINDER KAUR
Direct antiproliferative effect of cyclosporin A on cultured keratinocytes and fibroblasts 693
B.J.NICKOLOFF AND R.S.MITRA
Persistent bite reactions responsive to photochemotherapy 693
B.E.BEACHAM AND D.KURGANSKY

Book Review 695

News and Notices 696

No. 6: DECEMBER 1990

Langerhans cells but not monocytes are capable of antigen presentation *in vitro* in corticosteroid contact hypersensitivity ... 699
A.I.LAUERMA, L.RÄSÄNEN, T.REUNALA AND S.REITAMO
The cytokeratin expression of cultured human foetal keratinocytes ... 707
ANNE M.OLIVER
Expression of extracellular matrix in hair follicle mesenchyme in alopecia areata ... 717
A.J.G.MCDONAGH, LISA CAWOOD AND A.G.MESSENGER
Silica-associated systemic sclerosis is clinically, serologically and immunologically indistinguishable from idiopathic systemic sclerosis ... 725
M.H.A.RUSTIN, HELEN A.BULL, V.ZIEGLER, J.MEHLHORN, U.F.HAUSTEIN, P.J.MADDISON, JUNE JAMES AND PAULINE M.DOWD
Production of antikeratin autoantibodies by hybrid spleen cells of naive mice ... 735
K.IWATSUKI, S.IMAIZUMI, H.HASHIZUME, K.SUGAYA, M.TAKIGAWA AND M.YAMADA
The changing face of dermatology out-patient referrals in the south-east of Scotland ... 745
D.W.S.HARRIS, E.C.BENTON AND J.A.A.HUNTER
Validation of Sickness Impact Profile and Psoriasis Disability Index in psoriasis ... 751
A.Y.FINLAY, G.K.KHAN, D.K.LUSCOMBE AND M.S.SALEK
Pigmented spindle cell naevus ... 757
L.REQUENA AND E.SANCHEZ YUS
Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare ... 765
BRONWEN CHAMBERS, A.MILLIGAN AND A.FLETCHER
Anxiety and depression in patients with chronic urticaria and generalized pruritus ... 769
R.A.SHEEHEN-DARE, M.J.HENDERSON AND J.A.COTTERILL
Clear ultraviolet blocking lenses for use by PUVA patients ... 775
H.MOSELEY AND S.K.JONES
Factor XIIIa in nodular malignant melanoma and Spitz naevi ... 783
KAREN J.DENTON, D.W.K.COTTON, A.WRIGHT AND PATRICIA HIRD
Topical treatment of necrotic foot ulcers in diabetic patients: a comparative trial of DuoDerm and MeZinc ... 787
J.APELQVIST, J.LARSSON AND A.STENSTRÖM
Perianal streptococcal cellulitis with penile involvement ... 793
P.DUHRA AND A.ILCHYSHYN
Erythema multiforme major in a 2-month-old child with human immunodeficiency virus (HIV) infection ... 797
D.SALOMON AND J-H.SAURAT
Necrolytic migratory erythema: association with neuroendocrine tumour with predominant insulin secretion 801
S.M.WILKINSON, P.H.CARTWRIGHT, C.ALLEN, S.REEVES, L.ALEXANDER AND J.P.H.BYRNE
Angiotropic B-cell lymphoma (malignant angioendotheliomatosis): failure of systemic chemotherapy ... 807
R.E.A.WILLIAMS, MORAG M.SEYWRIGHT, ROSEMARY LEVER AND N.P.LUCIE

Society Proceedings

British Society for Investigative Dermatology Annual Meeting, Newcastle, September 1990 ... 811

Correspondence

Increased frequency of HLA-DR2 in patients with Darier's disease ... 845
ANNEMETTE OXHOLM, P.OXHOLM, F.DA CUNHA BANG, J.U.PRAUSE, N.ODUM, B.K.JAKOBSEN AND A.SVEJGAARD
Sensitization as a result of a religious ritual ... 846
A.J.CARMICHAEL AND I.S.FOULDS
Varicella bullosa in an adult ... 846
M.YOSHIDA, S.KUSUDA AND T.TEZUKA

News and Notices ... 849

Index ... 851

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 123, Number 6, December 199

CONTENTS PAGE

Langerhans cells but not monocytes are capable of antigen presentation *in vitro* in corticosteroid contact hypersensitivity ... 699
A.I.LAUERMA, L.RÄSÄNEN, T.REUNALA AND S.REITAMO

The cytokeratin expression of cultured human foetal keratinocytes ... 707
ANNE M.OLIVER

Expression of extracellular matrix in hair follicle mesenchyme in alopecia areata ... 717
A.J.G.MCDONAGH, LISA CAWOOD AND A.G.MESSENGER

Silica-associated systemic sclerosis is clinically, serologically and immunologically indistinguishable from idiopathic systemic sclerosis ... 725
M.H.A.RUSTIN, HELEN A.BULL, V.ZIEGLER, J.MEHLHORN, U-F.HAUSTEIN, P.J.MADDISON, JUNE JAMES AND PAULINE M.DOWD

Production of antikeratin autoantibodies by hybrid spleen cells of naive mice ... 735
K.IWATSUKI, S.IMAIZUMI, H.HASHIZUME, E.SUGAYA, M.TAKIGAWA AND M.YAMADA

The changing face of dermatology out-patient referrals in the south-east of Scotland ... 745
D.W.S.HARRIS, E.C.BENTON AND J.A.A.HUNTER

Validation of Sickness Impact Profile and Psoriasis Disability Index in psoriasis ... 751
A.Y.FINLAY, G.K.KHAN, D.K.LUSCOMBE AND M.S.SALEK

Pigmented spindle cell naevus ... 757
L.REQUENA AND E.SANCHEZ YUS

Epidermal dendritic S100 positive cells in necrobiosis lipoidica and granuloma annulare ... 765
BRONWEN CHAMBERS, A.MILLIGAN AND A.FLETCHER

Anxiety and depression in patients with chronic urticaria and generalized pruritus ... 769
R.A.SHEEHEN-DARE, M.J.HENDERSON AND J.A.COTTERILL

Clear ultraviolet blocking lenses for use by PUVA patients ... 775
H.MOSELEY AND S.K.JONES

Factor XIIIa in nodular malignant melanoma and Spitz naevi ... 783
KAREN J.DENTON, D.W.K.COTTON, A.WRIGHT AND PATRICIA HIRD

Topical treatment of necrotic foot ulcers in diabetic patients: a comparative trial of DuoDerm and MeZinc ... 787
J.APELQVIST, J.LARSSON AND A.STENSTRÖM

Perianal streptococcal cellulitis with penile involvement ... 793
P.DUHRA AND A.ILCHYSHYN

Erythema multiforme major in a 2-month-old child with human immunodeficiency virus (HIV) infection ... 797
D.SALOMON AND J-H.SAURAT

Necrolytic migratory erythema: association with neuroendocrine tumour with predominant insulin secretion 801
S.M.WILKINSON, P.H.CARTWRIGHT, C.ALLEN, S.REEVES, L.ALEXANDER AND J.P.H.BYRNE

Angiotropic B-cell lymphoma (malignant angioendotheliomatosis): failure of systemic chemotherapy ... 807
R.E.A.WILLIAMS, MORAG M.SEYWRIGHT, ROSEMARY LEVER AND N.P.LUCIE

Society Proceedings

British Society for Investigative Dermatology Annual Meeting, Newcastle, September 1990 ... 811

Correspondence

Increased frequency of HLA-DR2 in patients with Darier's disease ... 845
ANNEMETTE OXHOLM, P.OXHOLM, F.DA CUNHA BANG, J.U.PRAUSE, N.ODUM, B.K.JAKOBSEN AND A.SVEJGAARD

Sensitization as a result of a religious ritual ... 846
A.J.CARMICHAEL AND I.S.FOULDS

Varicella bullosa in an adult ... 846
M.YOSHIDA, S.KUSUDA AND T.TEZUKA

News and Notices ... 849

Inde[illegible] ... 851